

Managing cover crops and nitrogen fertilization to enhance sustainability of sorghum cropping  
systems in eastern Kansas

by

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## Abstract

Growing cover crops (CCs) in rotation with cash crops has become popular in recent years for their many agroecosystem benefits, such as influencing nutrient cycling and reducing nutrient losses. This study aimed to (i) determine the long-term effects of no-till with CCs and varying nitrogen (N) rates on subsequent sorghum [*Sorghum bicolor* (L.) Moench] yield and yield components, (ii) assess how CCs affect the N dynamic in the soil-crop relationship during the growing season and N use efficiency (NUE) of sorghum, and (iii) define and evaluate important periods of nitrous oxide (N<sub>2</sub>O) losses throughout the cropping system. Field experiments were conducted during the 2014-15 and 2015-16 growing season in a three-year no-till winter wheat (*Triticum aestivum* L.) – sorghum – soybean [*Glycine max* (L.) Merr] rotation. Fallow management consisted of a chemical fallow (CF) control plus four CCs and a double-crop soybean (DSB) grown after wheat harvest. Nitrogen fertilizer was subsurface banded at five rates (0, 45, 90, 135, and 180 kg ha<sup>-1</sup>) after sorghum planting. On average, DSB and late-maturing soybean (LMS) provided one-third and one-half of the N required for optimum economic grain yield (90 kg N ha<sup>-1</sup>), respectively; resulting in increased grain yield when compared to the other CCs and CF with 0-N application. Crimson clover (*Trifolium incarnatum* L.) and daikon radish (*Raphanus sativus* L.) had no or negative effects on sorghum yield and N uptake relative to CF across all N rates. Sorghum-sudangrass (SS) (*Sorghum bicolor* var. sudanese) significantly reduced N uptake and grain yield, even at higher N rates. Sorghum following CF had the lowest NUE at optimum grain yield when compared to all CC treatments, suggesting that CCs have a tendency to improve NUE. Cover crops reduced N<sub>2</sub>O emissions by 65% during the fallow period when compared to CF; however, DSB and SS increased emissions when N was applied during the sorghum phase, indicating that N fertilization might be the overriding factor. Moreover, about 50% of the total N<sub>2</sub>O emissions

occurred within 3 weeks after N application, regardless of the cover crop treatment, indicating the importance of implementing N management strategies to reduce N<sub>2</sub>O emissions early in the growing season. Overall, these results show that CC selection and N fertilizer management can have significant impacts on sorghum productivity and N<sub>2</sub>O emissions in no-till cropping systems.

## Table of Contents

List of Figures .....	viii
List of Tables .....	xiii
Acknowledgements .....	xv
Dedication .....	xvi
Chapter 1 - Literature review .....	1
General introduction: The need of sustainable food production.....	1
Grain sorghum production .....	3
Overview .....	3
Grain sorghum production in Kansas .....	3
Nitrogen fertilizer and crop production .....	4
Nitrogen behavior in soils .....	6
Soil organic nitrogen .....	6
Soil inorganic nitrogen.....	7
Mechanisms of nitrogen loss in agricultural soils .....	9
Ammonia volatilization .....	9
Leaching.....	10
Denitrification .....	11
Nitrous oxide emissions and agriculture.....	14
Overview of greenhouse gas emissions .....	14
Nitrous oxide emissions in agricultural soils .....	15
Strategies for nitrous oxide mitigation in crop production .....	16
Role of cover crops in cropping systems .....	19
Benefits of cover crops .....	20
Nitrogen management .....	21
Improving crop yield.....	23
Nitrogen use efficiency in crop production .....	24
Definition and estimations of nitrogen use efficiency in plants.....	25
Improving nitrogen use efficiency .....	27

Project hypothesis and objectives .....	28
References .....	30
Chapter 2 - No-till grain sorghum response to cover crop and nitrogen fertilization.....	42
Abstract .....	42
Introduction.....	43
Material and methods.....	46
Site description and experimental design.....	46
Winter wheat and cover crop management.....	47
Grain sorghum management .....	48
Components of nitrogen use calculations .....	50
Nitrogen fertilizer replacement value .....	51
Statistical analysis .....	51
Results.....	52
Weather condition and soil analysis .....	52
Cover crop biomass and nitrogen production .....	52
In-season leaf nitrogen status of sorghum.....	53
Sorghum grain yield, dry matter, and nitrogen uptake .....	55
Components of nitrogen use efficiency .....	56
Nitrogen fertilizer replacement value .....	58
Discussion .....	59
Cover crop biomass and nitrogen production .....	59
In-season nitrogen leaf status of sorghum.....	60
Grain yield, dry matter, and nitrogen uptake .....	62
Components of nitrogen use efficiency .....	64
Nitrogen fertilizer replacement value .....	65
Conclusions.....	66
References.....	68
Chapter 3 - Replacing chemical fallow with cover crops in no-till cropping systems: Effects on nitrous oxide emissions.....	97
Abstract .....	97
Introduction.....	98

Material and methods.....	101
Site description and experimental design.....	101
Cropping system management.....	102
Winter wheat phase.....	102
Cover crop phase.....	102
Grain sorghum phase .....	103
Soil nitrous oxide emission measurements .....	105
Daily and cumulative nitrous oxide flux calculation .....	106
Yield-scaled nitrous oxide emissions and fertilizer induced emission factor .....	107
Ancillary measurements.....	107
Statistical analysis .....	108
Results.....	109
Weather condition.....	109
Winter wheat phase.....	109
Cover crop phase.....	110
Grain sorghum phase .....	112
Daily and cumulative nitrous oxide emissions .....	112
Yield parameters and nitrogen utilization efficiency .....	113
Cropping system cumulative and yield-scaled nitrous oxide emissions, and fertilizer induced emission factor .....	114
Discussion.....	115
Residual effects of cover crops on the winter wheat phase of the crop rotation.....	115
Role of cover crops on reducing nitrogen losses in the fallow period .....	117
Nitrous oxide emissions during the grain sorghum phase .....	118
Cropping system effect on nitrous oxide emissions .....	120
Conclusion .....	122
References.....	124
Chapter 4 - General conclusion.....	147
Appendix A - Assessment of nutrient and pH dynamic after eight years of no-till and cover crops .....	151
Appendix B - SAS code.....	171

## List of Figures

- Figure 2.1. Daily maximum and minimum temperature, and precipitation during the sorghum grain growing season in 2014 (A) and 2015 (B). (GS, growth stage; N, nitrogen; NDVI, Normalized Difference Vegetation Index; SPAD, chlorophyll index; PM, physiological maturity)..... 72
- Figure 2.2. Normalized Difference Vegetation Index (NDVI) values at growth stage 3 as affected by cover crop and nitrogen (N) fertilizer rate on sorghum grown in 2014 (A) and 2015 (B). Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.). ..... 73
- Figure 2.3. Interaction effects of cover crop by nitrogen (N) rate (A) and cover crop by year (B) on Normalized Difference Vegetation Index (NDVI) values at growth stage 5 of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.). ..... 74
- Figure 2.4. Relationship between sorghum grain yield and Normalized Difference Vegetative Index (NDVI) at growth stage 3 in 2014 (A), growth stage 5 in 2014 (B), growth stage 3 in 2015 (C), growth stage 5 in 2015 (D). (GS, growth stage)..... 75
- Figure 2.5. Relationship between total nitrogen (N) uptake at physiological maturity and Normalized Difference Index Vegetation (NDVI) at growth stage 3 in 2014 (A), growth stage 5 in 2014 (B), growth stage 3 in 2015 (C), growth stage 5 in 2015 (D). (GS, growth stage). ..... 76
- Figure 2.6. Leaf chlorophyll index as affected by cover crop and nitrogen (N) rates in sorghum grown in 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.). ..... 77



Figure 2.7. Relationship between leaf chlorophyll index and total nitrogen (N) uptake at physiological maturity (A) and sorghum grain yield (B) in 2015.....	78
Figure 2.8. Effects of cover crop and nitrogen (N) rate on sorghum grain yield across both years. Vertical bars indicate the 95% confidence interval. ....	79
Figure 2.9. Relationship between grain yield and total nitrogen (N) supplied (A) and total N uptake (B) on sorghum grown in 2014 and 2015. (Total N supplied was estimated as the N fertilizer applied plus N supplied by the soil). ....	80
Figure 2.10. Interaction effects of cover crop by nitrogen (N) rate (A) and N rate by year (B) on the average dry mater production of sorghum in 2014 and 2015 Vertical bars indicate the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.). ....	81
Figure 2.11. Effects of cover crop (A) and nitrogen (N) rates (B) on total N uptake at physiological maturity of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.). ....	82
Figure 2.12. Effects of cover crop by year (A) and nitrogen (N) rate by year (B) on N use efficiency of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.). ....	83
Figure 2.13. Interaction effects of cover crop by nitrogen (N) rate (A) and cover crop by year (B) on N utilization efficiency of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.). ....	84
Figure 2.14. Nitrogen (N) harvest index of sorghum as affected by cover crops and N rates across both years. Vertical bars indicate the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; CF, chemical fallow; DSB, double-crop soybean; LMS,	

late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).	85
Figure 2.15. Interaction effects of cover crop by nitrogen (N) rate (A) and cover crop by year (B) on partial factor productivity of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 45-N, 45 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 135-N, 135 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).	86
Figure 3.1. Mean daily nitrous oxide (N <sub>2</sub> O) emissions during the 2014-15 winter wheat phase of the rotation under different fallow and nitrogen (N) management. (A, 0 kg N ha <sup>-1</sup> ; B, 90 kg N ha <sup>-1</sup> ; C, 180 kg N ha <sup>-1</sup> applied during the 2012 sorghum phase of the crop rotation. Previous cover crops and double-crop soybean were grown after wheat harvest in 2011.).	128
Figure 3.2. Mean daily nitrous oxide (N <sub>2</sub> O) emissions during the 2015-16 winter wheat of the rotation under different fallow and nitrogen (N) management. (A, 0 kg N ha <sup>-1</sup> ; B, 90 kg N ha <sup>-1</sup> ; C, 180 kg N ha <sup>-1</sup> applied during the 2013 sorghum phase of the crop rotation. Previous cover crops and double-crop soybean were grown after wheat harvest in 2012. NS, not significant.).	129
Figure 3.3. Residual effects of fallow management on total cumulative N <sub>2</sub> O emissions from winter wheat planting to harvest in the 2014-15 and 2015-16 winter wheat phase of the rotation at Ashland Bottoms, KS. Vertical bars indicate Fisher's LSD at $P \leq 0.05$ . (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).	130
Figure 3.4. Mean daily nitrous oxide (N <sub>2</sub> O) emissions under different cover crop treatments during the 2014-15 (A) and 2015-16 (B) fallow period of the crop rotation at Ashland Bottoms, KS. (CCs, cover crops; CF, chemical fallow; DS, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish. First frost was the first day that minimum temperature was below 0°C.).	131
Figure 3.5. Total cumulative nitrous oxide (N <sub>2</sub> O) emissions as affected by cover crop treatments (A) and growing season (B) at the end of the fallow period of the rotation at Ashland	

Bottoms, KS. Vertical bars indicate Fisher's LSD at $P \leq 0.05$ . (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).	132
Figure 3.6. Soil nitrate concentration at the 0-5 cm depth in plots under different fallow management during the fallow period of 2015-16. (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).	133
Figure 3.7. Mean soil nitrous oxide ( $N_2O$ ) emissions as affected by different fallow management and nitrogen (N) rates during the 2015 grain sorghum phase of the rotation at Ashland Bottoms, KS. (A; 0 kg N ha <sup>-1</sup> ; B, 90 kg N ha <sup>-1</sup> ; C, 180 kg N ha <sup>-1</sup> ; N fertilizer application with 28% Urea Ammonium Nitrate.).	134
Figure 3.8. Mean total cumulative nitrous oxide ( $N_2O$ ) emissions during the grain sorghum growing season in 2015 as affected by nitrogen (N) fertilizer rate (A) and cover crop treatments (B). Vertical bars indicate the Fisher's LSD at $P \leq 0.05$ . (0-N; 0 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> .).	135
Figure 3.9. Grain yield response to cover crops and nitrogen (N) fertilization of sorghum grown in 2015 at Ashland Bottoms, KS. Vertical error bars indicate the 95% confidence interval.	136
Figure 3.10. Nitrogen (N) utilization efficiency of grain sorghum as affected by cover crop and N rate. Vertical bars indicate Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 90-N, kg N ha <sup>-1</sup> ; 180-N, kg N ha <sup>-1</sup> ; CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).	137
Figure 3.11. Dynamic of cumulative nitrous oxide ( $N_2O$ ) emissions under different cover crop (A) and nitrogen (N) rate treatments (B) from cover crop planting in 2014 through grain sorghum harvesting in 2015 at Ashland Bottoms, KS. Vertical bar at the last sampling event in B indicates the Fisher's LSD at $P \leq 0.05$ . (0-N; 0 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; NS, not significant.).	138
Figure 3.12. Relationship between nitrogen (N) fertilizer rate and total cumulative nitrous oxide ( $N_2O$ ) emission (A) and yield-scaled $N_2O$ emission. Total $N_2O$ emissions represent values from cover crop planting in 2014 through sorghum harvesting in 2015. (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).	139
Figure 3.13. Relationship between nitrogen (N) utilization efficiency and total cumulative nitrous oxide ( $N_2O$ ) emission (A) and yield-scaled $N_2O$ emission. Total $N_2O$ emissions represent	

values from cover crop planting in 2014 through sorghum harvesting in 2015. (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).	140
Appendix A. 1. Effects of fallow management on pH distribution with depth in 2014 (A) and 2015 (B). Horizontal bars indicate the Fisher's LSD at $P \leq 0.05$ . (NS, not significant.)	161
Appendix A. 2. Vertical distribution of pH in the soil profile as affected by nitrogen (N) management. Horizontal bar indicates the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 45-N, 45 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 135-N, 135 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; NS, not significant.)	162
Appendix A. 3. Effects of fallow management on mehlisch-3 phosphorus (P) concentration in the soil profile in 2014 (A) and 2015 (B). Horizontal bars indicate the Fisher's LSD at $P \leq 0.05$ . (NS, not significant.)	163
Appendix A. 4. Effects of fallow management on exchangeable potassium (K) concentration in the soil profile in 2014 (A) and 2015 (B). Horizontal bars indicate the Fisher's LSD at $P \leq 0.05$ . (NS, not significant.)	164
Appendix A 5. Vertical distribution of ammonium-N (NH <sub>4</sub> <sup>+</sup> ) in the soil profile as affected by cover crops in 2014 (A) and 2015 (B). Horizontal bars at each depth indicate the Fisher's LSD at $P \leq 0.05$ . (NS, not significant.)	165
Appendix A. 6. Mean nitrate-N ( ) concentration in the soil profile as affected by fallow and nitrogen (N) management. Horizontal bars at each depth indicate the Fisher's LSD at $P \leq 0.05$ . (A, 0 kg N ha <sup>-1</sup> ; B, 45 kg N ha <sup>-1</sup> ; C, 90 kg N ha <sup>-1</sup> ; D, 135 kg N ha <sup>-1</sup> ; E, 180 kg N ha <sup>-1</sup> ; NS, not significant.)	166
Appendix A. 7. Total nitrogen (N) concentration with soil depth as affected by fallow (A and B) and nitrogen (N) management (C and D) in 2014 and 2015, respectively. Horizontal bars at each depth indicate the Fisher's LSD at $P \leq 0.05$ . (0-N, 0 kg N ha <sup>-1</sup> ; 45-N, 45 kg N ha <sup>-1</sup> ; 90-N, 90 kg N ha <sup>-1</sup> ; 135-N, 135 kg N ha <sup>-1</sup> ; 180-N, 180 kg N ha <sup>-1</sup> ; NS, not significant.)	167
Appendix A. 8. Vertical distribution of soil organic carbon (C) in the soil profile as affected by cover crops. Horizontal bars at each depth indicate the Fisher's LSD at $P \leq 0.05$ . (NS, not significant.)	168

## List of Tables

Table 1.1. Total biomass production and nitrogen (N) uptake by cover crops grown in different environments and soil conditions.....	38
Table 1.2. Definitions and methods of estimating the efficiency of nitrogen (N) use.....	39
Table 1.3. Cereal production, nitrogen (N) use on cereals, and N use efficiency (NUE) by world region (average for the 1999 to 2003 period). ....	41
Table 2.1. Average monthly temperature and precipitation at Ashland Bottoms Research Farm in 2013, 2014, and 2015.....	87
Table 2.2. Selected soil chemical properties at the 15-cm depth taken during the spring before sorghum planting in 2014 and 2015.....	88
Table 2.3. Total aboveground dry matter production, nitrogen (N) content, N concentration, and carbon to N ratio (C:N) of summer and winter cover crops at termination date. ....	89
Table 2.4. ANOVA significance levels for the effect of cover crops, nitrogen rate, year, and their interactions on Normalized Difference Vegetation Index (NDVI) at Stage 3 and 5, and leaf chlorophyll index of grain sorghum grown in 2014 and 2015.....	90
Table 2.5. ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, year, and their interactions on grain yield, dry matter production, and total N uptake of sorghum grown in 2014 and 2015. ....	91
Table 2.6. Apparent nitrogen mineralization§ from each cover crop treatment during the sorghum growing season in 2014 and 2015.....	92
Table 2.7. ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, year, and their interactions on partial factor productivity (PFP), N use efficiency (NUE), N utilization efficiency (NUtE), N uptake efficiency (NUpE), N recovery efficiency (N <sub>RE</sub> ) and N harvest index (NHI) of grain sorghum grown in 2014 and 2015. ....	93
Table 2.8. Main effects of the cover crop, nitrogen (N) rates, and year on N uptake efficiency on sorghum grown in 2014 and 2015 in Ashland Bottoms, KS. ....	94
Table 2.9. Regression equations of sorghum grain yield as a function of nitrogen fertilizer rate for the chemical fallow treatment in 2014 and 2015. ....	95
Table 2.10. Estimated nitrogen fertilizer replacement value provided to grain sorghum by cover crops and double-crop soybean in 2014 and 2015.....	96

Table 3.1. Average monthly temperature and precipitation at Ashland Bottoms Research Farm in 2014, 2015, and 2016.....	141
Table 3.2. ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, date, and their interaction on daily nitrous oxide emissions during the wheat, cover crop, and sorghum phases of the rotation. ....	142
Table 3.3. ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, year, and their interactions on total cumulative nitrous oxide emissions at the end of the wheat, cover crop, and sorghum phases of the rotation. ....	143
Table 3.4. Effects of cover crop (CC) and nitrogen (N) rate treatments on total aboveground dry matter yield, total N uptake, and grain N yield of sorghum grown in 2015 at Ashland Bottoms, KS.....	144
Table 3.5. Effects of cover crop (CC) and nitrogen (N) rate treatments on total cumulative nitrous oxide (N <sub>2</sub> O) emissions at the end of the 2014-15 cover crop and 2015 sorghum phase, and the combined cover crop plus sorghum period. ....	145
Table 3.6. Effects of cover crop (CC) and nitrogen (N) rate treatments on three different indexes of yield-scaled N <sub>2</sub> O emissions* (YSNE) and fertilizer induced emission factor* (FIEF) of sorghum grown in 2015 at Ashland Bottoms, KS. ....	146
Table A. 1. Summary of the ANOVA significance levels within soil depth for pH, Mehlich-3 phosphorus (P), exchangeable potassium (K), nitrate-N (NO <sub>3</sub> <sup>-</sup> ), ammonium-N (NH <sub>4</sub> <sup>+</sup> ), total nitrogen (N), and total organic carbon (C).....	169

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## **Dedication**

To my family.



# Chapter 1 - Literature review

## General introduction: The need of sustainable food production

Recent studies have estimated that by 2050 the world population will increase from 7 billion people today to approximately 9 billion, adding pressure to the food supply system (Godfray et al., 2010). Furthermore, farmland in food production has been switching over to biofuel production, and thus impacting total food supply (Godfray, 2014). In this sense, the main challenge for global agriculture for the next decades will be to meet the demand for food, fiber and energy while maintaining the sustainability of the agro-ecosystems (Killham, 2011). Therefore, it is necessary that the increase in food production rely on the further increase in yield of current agricultural land while minimizing environmental consequences.

Nitrogen (N) fertilizers play important roles in agricultural production, especially in staple food crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and grain sorghum [*Sorghum bicolor* (L.) Moench]. In fact, as much as two-fifths of the world's population would not be alive today without the industrial process of fixing N from the atmosphere to make fertilizer (Smil, 2004). According to Tilman et al. (2001), the doubling of global food production during the past 35 years was accompanied by a seven-fold increase in N fertilization, from 12 million metric ton (MT) in 1965 to 87 million MT in 2000. However, the increased use of inorganic N fertilizers has also led to a host of environmental problems, such as eutrophication, groundwater pollution, and nitrous oxide (N<sub>2</sub>O) emissions from agricultural lands (Gruber and Galloway, 2008).

Nitrogen is an essential nutrient to crop plants, and it is often the nutrient that most limits yield. Retail prices range from \$0.55 to \$0.85 kg<sup>-1</sup> for most commonly used N fertilizers (USDA-ECS, 2013). Application costs usually drive the total price of N fertilizer even higher. Thus,

depending on the amount required and the source of fertilizer, a farmer could spend up to \$110 acre<sup>-1</sup> for N. Furthermore, recovery of applied N fertilizer is usually less than 50% worldwide, and the dynamic nature of N in soil makes it more susceptible to loss by volatilization, leaching, and denitrification (Fageria and Baligar, 2005). Nitrogen fertilizer can be expensive, inefficient, environmentally damaging, and hard to manage, thus, it is important to find other means to supply and retain adequate levels of soil mineral N for crop production.

The use of cover crops (CCs) has been proposed as an alternative method to enhance the sustainability of cropping systems by improving environmental quality and reducing the negative effects of continuous cropping systems (Boer et al., 2007). One of the major benefits of CCs is the ability to improve nutrient cycling and reduce N loss. Depending on the specie, these plants can take up nutrients from deep soil layers or fix atmospheric N through symbiotic relationships with N-fixing bacteria and release them later on the soil surface as the residue decomposes. It has been estimated that crops grown in fields after legume CCs can take up 30 to 60% of the N produced by the cover crop (Sarrantonio and Gallandt, 2003), thereby decreasing the amount of synthetic N fertilizer needed for the next cash crop.

In face of the above, the sustainability of food production could be achieved by the use of agronomic practices that increase soil productivity, crop yield, and water and nutrient use efficiency. Such practices are: conservation tillage, crop rotation with leguminous and non-leguminous species, cover cropping, integrated nutrient management with use of manure and compost, etc. The combination of these methods can be the key to sustainable intensification and food security (Godfray, 2014).

## **Grain sorghum production**

### ***Overview***

Sorghum ranks fifth in world cereal production after wheat, corn, rice, and barley (*Hordeum vulgare* L.) (FAOSTAT, 2015). In general, sorghum is produced across the world in the warmer climatic areas (39% in Africa, 38% in America, 17.7% in Asia, 1.5% in Europe, and 3.8% in Oceania) (FAOSTAT, 2015). In the last 5 years, the annual global production ranged from 55 to 60 million MT, with an annual yield growth rate of 3.6% (FAOSTAT, 2015).

Although the United States had a negative annual yield growth rate of 6.38% in the last 5 years, the country is still the major producer worldwide followed by Mexico, India, Nigeria, and Sudan (FAOSTAT, 2015). Most of the sorghum in industrialized countries is used as an ingredient in animal feed rations. In Asia and Africa, sorghum is produced for human consumption, and the production is largely based on small-scale farming (Kleih et al., 2000). In developing countries, sorghum is an essential component of the cropping systems and plays an important role in the diet, particularly in semi-arid tropical regions where drought can cause frequent failure of other crops.

### ***Grain sorghum production in Kansas***

Agriculture is the largest economic driver in Kansas, valued at more than \$62 billion and accounting for 43% of the state's total economy (Kansas Department of Agriculture, 2015). Grain sorghum is well adapted in most parts of Kansas due to two main characteristics: tolerance of drought and high temperature. These tolerances allow sorghum to be grown in regions with high summer temperature and low precipitation where other crops are more likely to fail or become unprofitable (Mahama et al., 2015).

Kansas is the largest producer of grain sorghum in the United States, supplying 42 and 46% of the total production in 2013 and 2014, respectively. In 2013, Kansas produced approximately

4.27 million MT, while in 2014 the total production was estimated in 5.08 million MT, representing an increase of 18% from the previous year (USDA-NASS, 2015). The average yield in Kansas for the last 5 years ranged from 2.45 to 4.65 Mg ha<sup>-1</sup> (Mg = 10<sup>6</sup> g) (USDA-NASS, 2015). The year-to-year variability of sorghum yield can be explained by the many abiotic (chemical and physical soil properties, weather patterns, etc.) and biotic factors (weed, insects, disease, etc.) that influence plant growth and development.

### **Nitrogen fertilizer and crop production**

The atmosphere is considered to be the biggest pool of N on the planet, comprised of about 78% dinitrogen (N<sub>2</sub>). However, this form of N is not readily available for plant uptake. There are two forms of bacteria that have the ability to fix atmospheric N<sub>2</sub> into an N form that can be used as a source of energy for metabolic functions: they are (i) free living bacteria (or non-symbiotic bacteria) that live throughout the soil, and (ii) mutualistic bacteria (or symbiotic) that live in nodules in the roots of certain plants. Because of the importance of N to higher life forms, some plants have developed symbiotic relationships with N-fixing bacteria that allow them access to N fixed from the air (Scharf, 2015).

Nitrogen is one of the major essential nutrients because it is required in large amounts and has many functions in the growth and development of plants. Therefore, production of food and fiber has been limited more by availability of N than by that of any other essential element (Aulakh et al., 1992). The world N fertilizer consumption in 2010 was estimated at approximately 104 million MT (FAOSTAT, 2015). Out of this total, 46.2% was applied on corn, wheat, and rice, and only 4.6% was used on other cereals such as sorghum, barley, oats (*Avena sativa* L.), pearl millet [*Pennisetum americanum* (L.) Leek], rye (*Secale cereal* L.), and triticale (*Tricale hexaploid* L.) (Heffer, 2009). In fact, Stewart et al. (2005) estimated that in the United States, N fertilizer was

responsible for increasing yields of corn, rice, sorghum, and wheat by 41, 27, 19, and 16%, respectively.

The increased use of N fertilizer has resulted in dramatic yield gaps of the major cereal crops (Krupnik et al., 2004). In the past, biological N fixation was the chief means of supplying N for cultivated crops, but farmers can no longer rely on soil reserves from previous crops which had biological N fixation to meet the N requirement to maintain and improve crop yield (Stevenson, 1982). Currently, the addition of synthetic N to sustain and increase crop yields is a universal and fundamental feature of modern crop management (Robertson and Vitousek, 2009). This is mainly due to the invention of the Haber-Bosch process, which is a chemical process to convert  $N_2$  to ammonia ( $NH_3$ ), which in turn is converted into commercial N fertilizers (Galloway and Cowling, 2002). Common fertilizers in crop production usually contain nitrogen in one or more of the following forms: nitrate ( $NO_3^-$ ),  $NH_3$ , ammonium ( $NH_4^+$ ), or urea ( $CO(NH)_2$ ) (Mengel, 1986).

As a cereal crop, grain sorghum needs substantial amounts of N; however, sorghum is considered a “low input crop” when compared to other crops. Thus, N management for grain sorghum production has received less attention and consequently limited yields. In the United States, less than 2.6% of the total N fertilizer used in 2010 was used on grain sorghum, but 49 and 13.1% were applied on corn and winter wheat, respectively (Heffer, 2009).

Although most of the crops obtain 50-80% of their N requirement from the soil, chemical fertilizers are important complementary sources for maximum economic yield (Fageria and Baligar, 2005). However, the average N recovery efficiency for cereal production is approximately 33%, and the unaccounted 67% represents about \$15.9 billion annual loss of N fertilizer (Raun and Johnson, 1999). Hence, erosion and leaching of agricultural lands with excess fertilizer are the major causes of surface and groundwater pollution (Aulakh et al., 1992). For this reason, the

importance of the efficient management of N as well as knowing and applying the correct rate is both economically and environmentally beneficial.

## **Nitrogen behavior in soils**

### ***Soil organic nitrogen***

Organic N is the dominant N form in nearly all soils (Scharf, 2015). Although organic N is not directly available to plants, it is still an important reservoir that supplies a substantial amount of the available N in agricultural systems (Scharf, 2015). In general, the amount of N fertilizer required by crops will vary depending on the expected yield, the amount of organic matter (OM), and residual N (inorganic forms of N) in the soil prior to planting.

Mineralization is the conversion of organic N (from OM and crop residues) to plant available forms of N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ). This process is performed by heterotrophic soil organisms that utilize nitrogenous organic substances as an energy source (Jansson and Persson, 1982). Because it is a living microbial process, the speed of mineralization depends mainly on soil moisture and temperature. The rule of thumb is that more N will be mineralized if the soil is warm, moist, and well-aerated throughout the growing season, thereby increasing its availability for plant uptake. This aspect is considered very important in sorghum production because it is a non-legume<sup>1</sup> summer crop. In Kansas, grain sorghum is usually planted from mid-May to early July, allowing sorghum to utilize N mineralized from residues and OM more effectively and reducing

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<sup>1</sup>In the botanical classification, the crop plants are divided into two distinct groups based on characteristics of inflorescence and fruits: the grasses family (Gramineae) and legume family (Leguminosae) (Fageria, 2009, pp. 12). A key characteristic of legume plants is the ability to establish a symbiotic relationship with bacteria species (*Rhizobium* and *Bradyrhizobium*) that are responsible for N fixation.

total N fertilizer requirement. However, the quantity and quality of the crop residues affects the N availability. Crop residues can either release or immobilize inorganic N.

Immobilization can be described as the opposite of mineralization and is the conversion of inorganic N into organic forms. Soil organisms assimilate inorganic N compounds and transform them into organic N constituents of their cells and tissues, called the soil biomass (Jansson and Persson, 1982). Both the mineralization and immobilization process are mainly driven by the residue quality, expressed as the carbon to nitrogen (C:N) ratio. In general, organic materials with low C:N ratio (< 25:1) will favor mineralization and N will be release. On the other hand, immobilization will occur and N will be unavailable for plant uptake if organic materials have C:N ratio higher than 25:1 (Jansson and Persson, 1982).

Under favorable conditions, residues of legume plants decompose faster than non-leguminous crops (corn and grain sorghum stalks, wheat straw, etc.) due to their low C:N ratio (~ 20:1), and increasing soil mineral N availability for plant uptake. In a study evaluating the effects of cropping sequence on sorghum, Yamoah et al. (1998) observed that, where no N fertilizer was applied, sorghum grain yield in rotation with soybeans [*Glycine max* (L.) Merr] was, on average, 26% higher compared to continuous sorghum, yielding up to 7.12 Mg ha<sup>-1</sup>. In the same study, the authors also observed that, on average, sorghum grown in rotation with soybean did not generally respond to N fertilizer, suggesting that high fertilizer N rates are unnecessary in rotation systems with legume crops, particularly in years with high temperature and high precipitation during the growing season.

### ***Soil inorganic nitrogen***

The N form of NH<sub>4</sub><sup>+</sup> formed from the mineralization process is converted to NO<sub>3</sub><sup>-</sup> by a process called nitrification. This process is carried out by a specialized group of bacteria called

“nitrifiers” that obtain their energy through the conversion. The process is comprised of two stages: the oxidation of  $\text{NH}_4^+$  to nitrite ( $\text{NO}_2^-$ ), and the subsequent oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (Schmidt, 1982). In fact, if environmental conditions are not limiting,  $\text{NH}_4^+$  is oxidized to  $\text{NO}_3^-$  almost as rapidly as it is formed (Schmidt, 1982). Because nitrification is a microbial process, like mineralization, it is dependent on soil properties such as temperature, moisture, pH, organic carbon, oxygen supply,  $\text{NH}_4^+$  availability, etc. In general, warm (above  $10^\circ\text{C}$ ), moist soils that are unsaturated (oxygen supply) provide favorable conditions for nitrification. For this reason, nitrification occurs rapidly during the spring and summer months, slows down in the fall, and is essentially zero during the winter. For these reasons,  $\text{NO}_3^-$  concentration in soils is higher than  $\text{NH}_4^+$  throughout most of the year.

Ammonium and nitrate are the main forms of inorganic N in soil and they are the only forms of N that can be taken up by plants. Of these two N forms,  $\text{NH}_4^+$  is preferred for plant uptake because it is readily converted to amino acids, which in turn is used to produce more complex molecules such as proteins, enzymes, etc. (Mengel et al., 2001). On the other hand, when  $\text{NO}_3^-$  is assimilated, the plant needs to spend energy to back transform  $\text{NO}_3^-$  into  $\text{NH}_4^+$  in order to produce new organic compounds.

The behaviors of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in soil are very different. Ammonium has a positive charge and is attracted to the negative charges of clay particles and OM, and thus does not leach as easily as  $\text{NO}_3^-$ . In contrast,  $\text{NO}_3^-$  has a net negative charge and tends to be repelled by clay particles and OM, therefore it is found mostly in the soil solution. In addition,  $\text{NO}_3^-$  is soluble, so as water moves through soil, it moves freely with the water. The downward movement of dissolved nutrients through the soil profile with percolating water is called leaching (Lehmann and Schroth, 2003).



## **Mechanisms of nitrogen loss in agricultural soils**

Nitrogen behavior in soil is complex and can involve fairly rapid conversion from one N form to another, with different characteristics (Scharf, 2015). When N fertilizer is applied to the soil, it rapidly undergoes chemical transformations and is exposed to different loss pathways. The main pathways of N loss from agricultural soil are through volatilization, leaching, denitrification, and surface runoff. For this literature review, leaching and denitrification are the main focus and will be more extensively discussed.

### ***Ammonia volatilization***

When urea from animal urine, or a urea-based fertilizer is applied to the soil, the first reaction is the breakdown of urea into  $\text{NH}_3$  by the urease enzyme (Scharf, 2015). This enzyme is found in essentially all soil and plant residues. Next,  $\text{NH}_3$  reacts with soil water to form  $\text{NH}_4^+$ . Urea is an uncharged molecule and very soluble. It tends to move with soil water and thus can be lost through leaching if not converted to  $\text{NH}_4^+$  (Mengel, 1986).

Ammonia is a gas at atmospheric pressure and it is found in equilibrium with  $\text{NH}_4^+$  and can be lost from the soil and return to the atmosphere. This process is called  $\text{NH}_3$  volatilization. If conditions favor a high proportion of  $\text{NH}_3$  gas in the equilibrium between  $\text{NH}_3$  and  $\text{NH}_4^+$ , volatilization loss can be high, especially when urea-based fertilizers are applied at the soil surface and not incorporated by either tillage or a precipitation/irrigation event (Scharf, 2015). The proportion of nitrogen lost from urea-based N fertilizers due to  $\text{NH}_3$  volatilization may range from 1 to more than 50%, depending on fertilizer type, environmental conditions (temperature, wind speed, and rain), and soil chemical properties (calcium content, cation exchange capacity, and acidity) (Sommer et al., 2004).

### *Leaching*

Since the 1970s, nitrate leaching (NL) from croplands has become a significant concern because of its direct impact on water quality (Rivett et al., 2008). In environments where precipitation is greater than evapotranspiration, the excess water will percolate downward through the soil profile, moving  $\text{NO}_3^-$  to groundwater (Scharf, 2015). In irrigated agriculture, excessive water application not only increases NL but also leads to a vicious circle where low crop N availability is compensated with higher N fertilizer rates, increasing the probability of groundwater pollution (Quemada et al., 2013).

The National Primary Drinking Water Regulation specifies that the  $\text{NO}_3\text{-N}$  levels in United States drinking water are not to exceed 10 parts per million (ppm) (USEPA, 2015a). According to the Environmental Protection Agency (EPA), infants below six months of age who drink water containing nitrate in excess of the maximum contaminant level could become seriously ill and, if untreated, may die. The toxicity of nitrate in humans results from the reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , which reacts with hemoglobin to form methemoglobin, a substance that does not bind and transfer oxygen to tissues, thereby causing asphyxia (Majumdar, 2003).

Fifty percent of the domestic water supply in the United States comes from groundwater (USEPA, 2015a). It has been estimated that about 4% of domestic wells exceed the  $\text{NO}_3\text{-N}$  limits in the United States. This occurs mainly in the Great Plains states, regions of intensive irrigated agriculture such as California's Central Valley, and areas of sandy or other well-drained soils (Scharf, 2015). For this reason, understanding the fate of nitrate leaching in soils to groundwater is essential for managing risks associated with nitrate pollution, and to safeguard groundwater supplies and groundwater-dependent surface waters (Rivett et al., 2008).

Even so, nitrate leaching from agricultural soils is a complex process that depends on many factors such as soil characteristics, climatic variables, and management aspects (Plaza-Bonilla et

al., 2015). This complexity still limits our ability to understand, model, predict, and manage NL (Scharf, 2015). In general, more leaching can be expected in sandy soils (i.e., coarse-textured soil) than in clayey soils (i.e., fine-textured soil), and on soils under irrigation than dryland conditions.

However, Keeney and Follet (1991) reported that in most cases where N fertilizer causes  $\text{NO}_3^-$  pollution, it is due to excessive application or poor management practices. In a meta-analysis evaluating strategies to control NL in irrigated agricultural systems, Quemada et al. (2013) observed that management practices that adjusted water application to crop needs reduced NL by 40%, and the best relationship between yield and NL was obtained when the recommended N fertilizer rate was applied.

Post-harvest NL is another major concern of N loss. Although microbial activity is reduced during the fall and winter, mineralization and subsequent nitrification processes are still occurring, thereby increasing mineral N concentration in soil. If the soil is left in fallow, for example, the excess  $\text{NO}_3^-$  accumulated in soil will be more susceptible to losses through leaching and/or denitrification. Growing CCs in rotation with cash crops has been shown to be an effective strategy to reduce NL because CCs take up post-harvest  $\text{NO}_3^-$  from soil and consequently prevent the escape (Scharf, 2015). Therefore, the replacement of chemical fallow with winter CCs in areas with surplus rainfall during winter and spring has become an essential practice in temperate climates (Lemaire et al., 2004). In the same meta-analysis cited above, Quemada et al. (2013) found that NL reduced on average 50% when chemical fallow was replaced with non-legume CCs.

### ***Denitrification***

Denitrification is another loss pathway for  $\text{NO}_3^-$  in soil and can be a mechanism of significant loss of fertilizer and soil N from agricultural fields (Scharf, 2015). Denitrification is a form of anaerobic bacterial respiration during which  $\text{NO}_3^-$  is progressively transformed into  $\text{N}_2$ ,

that is,  $\text{NO}_3^-$  is reduced sequentially through  $\text{NO}_2^-$ , nitric oxide (NO) and  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Aulakh et al., 1992). Each reduction in this process is performed by different enzymes called nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (Firestone, 1982). In other words, nitrate is converted to gaseous forms of N that can be highly susceptible to loss to the atmosphere.

Denitrification under field conditions is complex and difficult to manage because it is controlled by the interaction of many factors such as soil pH, texture, temperature, organic C and mineral N supply, soil oxygen ( $\text{O}_2$ ), as well as soil water status (Mosier et al., 2002). In general, denitrification rates will be higher in warm and wet soils because the microorganisms regulate the reactions, and they are temperature and pH sensitive. Most denitrifying bacteria have optimum growth in alkaline soils where pH ranges between 6 and 8, and they are more active in warm soils than in cold soils (Aulakh et al., 1992).

Soil water content can directly and indirectly influence denitrification through: (i) providing favorable conditions for microbial growth and activity; (ii) reducing  $\text{O}_2$  concentration by filling soil pores; and (iii) release available C and N substrates through wetting and drying cycles (Aulakh et al., 1992). Under anaerobic conditions or saturated soil, the bacteria responsible for denitrification synthesize a series of enzyme reductases that enable them to use successively more reduced N oxides as electron acceptors in the absence of oxygen (Firestone, 1982). However, nitrous oxide reductase appears to be more sensitive to oxygen than either nitrate or nitrite reductase (Mosier et al., 2002). Consequently, if oxygen is limiting, the bacteria will continue to reduce nitrate until  $\text{N}_2$  and thus  $\text{N}_2$  gas is emitted to the atmosphere. On the other hand, denitrification is not fully realized in more aerobic conditions and intermediate sub products (i.e.,

NO and N<sub>2</sub>O) can be lost from soils, accounting for a significant part of the total greenhouse gas (GHG) emissions to the atmosphere from agricultural lands.

Soil water-filled pore space (WFPS) is an index that measures the degree of water saturation in a given soil, and it is assumed to be a useful parameter to predict microbial activity and consequent loss of soil N through microbial denitrification (Doran et al., 1990). According to Linn and Doran (1984), it is expected that aerobic microbial activity and subsequent respiration and nitrification will peak at about 60% WFPS, whereas denitrification will increase significantly at greater than 80% of WFPS due to reduction of O<sub>2</sub> availability (i.e., anaerobic condition). Additionally, Mosier et al. (2002) concluded that, on average, the largest NO emissions could be expected at WFPS of 30 to 60%, the highest N<sub>2</sub>O emissions when WFPS ranges from 50 to 80%, and that N<sub>2</sub> should be the dominant gas emitted in WFPS higher than 80%.

Carbon availability also affects denitrification rates. Because the majority of denitrifying organisms are heterotrophs, they use organic C compounds as electron donors for energy and synthesis of cellular constituents (Aulakh et al., 1991). Therefore, denitrification is strongly dependent on the availability of organic compounds such as soil organic matter, crop residues, root exudates, etc., (Aulakh et al., 1992). However, the quality of the organic materials also influences denitrification rates. In a study evaluating the effects of different types of crop residues, Aulakh et al. (1991) found that the cumulative denitrification loss with time were, in general, inversely related to C:N ratio of residue, i.e., denitrification losses were increasing with decreasing residue C:N ratio. In a similar study, Senbayram et al. (2012) conducted an incubation experiment using biogas residue (low available C source), maize straw (C source with moderate availability), and sucrose (highly available C source) to test the effects of the quality of added C sources on

denitrification rates. Overall, the authors found that organic substrate with large amounts of labile C induced drastic increases in the denitrification rate.

## **Nitrous oxide emissions and agriculture**

### ***Overview of greenhouse gas emissions***

Global atmospheric concentrations of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and N<sub>2</sub>O have increased markedly as a result of human activities over the past century (IPCC, 2007a). From the pre-industrial era (i.e., starting about 1750) to 2005, concentrations of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O have increased globally by about 152, 43, and 20%, respectively (IPCC, 2007a). These three gases are considered the primary greenhouse gas (GHG) because they represent about 80% of the human-induced warming effects of all GHGs (Solomon et al., 2007).

Despite a growing number of climate change mitigation policies, annual GHG emissions grew on average by 2.2% per year from 2000 to 2010 compared to 1.3% per year from 1970 to 2000 (IPCC, 2014). In the United States, emissions of GHG have increased by 5.9% from 1990 to 2013, representing an average annual increase of 0.3% (USEPA, 2015b).

The majority of GHGs come from burning fossil fuels to produce energy, although deforestation, industrial processes, and some agricultural practices also emit gases into the atmosphere (USEPA, 2015c). Since 1970, about 6% of the global GHG emissions have been in the form of N<sub>2</sub>O, and most important, more than one-third of all N<sub>2</sub>O emissions are anthropogenic and primarily due to agriculture, mainly because the increase use of N fertilizer in crop production (IPCC, 2014). In the United States, 9% of the total GHG emissions in 2013 were from the agriculture sector, of which N<sub>2</sub>O accounted for about 5% (USEPA, 2015b). The agricultural impact comes mainly from agricultural soil management, resulting in 74% of the total N<sub>2</sub>O emissions (USEPA, 2015b).

### *Nitrous oxide emissions in agricultural soils*

The global atmospheric N<sub>2</sub>O concentration increased from a pre-industrial value of 270 to 319 parts per billion (ppb) in 2013 (IPCC, 2014). Even though N<sub>2</sub>O occurs at lower concentration than CO<sub>2</sub> in the atmosphere, N<sub>2</sub>O has a global warming potential 300 times higher than CO<sub>2</sub> (Solomon et al., 2007). This means that N<sub>2</sub>O is 300 times more powerful than CO<sub>2</sub> at absorbing heat in the atmosphere. In addition, N<sub>2</sub>O molecules stay in the atmosphere for an average of 114 years before being removed by a sink or destroyed through chemical reactions, causing a long-term influence on climate (Solomon et al., 2007; USEPA, 2015c).

Agricultural fields are the major sources of N<sub>2</sub>O worldwide (Bouwman et al., 2002). Nitrous oxide gas is produced in soil by microbial metabolism primarily during the process of denitrification and, to a lesser extent, during nitrification (Klefoth et al., 2012). Emissions of N<sub>2</sub>O from agriculture constitute about 50% of total global anthropogenic flux of N<sub>2</sub>O (IPCC, 2014).

It has been documented that N<sub>2</sub>O emissions are well correlated with moist and warm conditions, but other controlling factors such as N and C availability or microbial community dynamics also exert a significant control on the temporal dynamic of N<sub>2</sub>O fluxes (Luo et al., 2013). Furthermore, the addition of synthetic N fertilizer can be a major factor controlling N<sub>2</sub>O emissions from croplands (Shcherbak et al., 2014).

In agricultural soils, the rate of denitrification has primarily been determined by the application of N fertilizers because it has had a direct impact on soil mineral N availability (Bouwman et al., 2002). The increased mineral N in soil after fertilizer application can either be taken up by crops if it remained in the root zone, or be subject to loss through leaching and denitrification (Zhu and Chen, 2002). Thus, N fertilizer rate has an important impact on N<sub>2</sub>O production, particularly when fertilizer rates exceed the maximum recommended rate for a given crop yield goal (Snyder et al., 2009). In this case, applications in excess of plant uptake result in

surplus N in soils, which in turn increase denitrification rates and, consequently, N<sub>2</sub>O emissions. Based on this, as N input increases, the direct N<sub>2</sub>O emissions would be expected to increase linearly.

In a study conducted by Bouwman et al. (2002) to assess the influence of factors regulating N<sub>2</sub>O emissions, the authors summarized information from 846 measurements in agricultural fields and found a strong increase of N<sub>2</sub>O accompanying N application rates. In a similar study, Shcherbak et al. (2014) conducted a meta-analysis of 78 published studies and found that the N<sub>2</sub>O response to N inputs was exponential, that is, the N<sub>2</sub>O emissions increased progressively with increasing N fertilizer once the input exceeded crop N demand. Overall, response curves for N<sub>2</sub>O flux as a function of N rate are not common but could help to better predict region- and site-specific N<sub>2</sub>O emissions in response to N additions (Hoben et al., 2011).

Despite detrimental effects on N<sub>2</sub>O emissions and other losses of soil N to the environment, N fertilizer remains essential to global food production, and therefore, agricultural management practices should focus on lowering the risks of N losses in order to optimize fertilizer N use efficiency (NUE) under median rates of N input, rather than minimize N application rates (Van Groenigen et al., 2010).

### ***Strategies for nitrous oxide mitigation in crop production***

It has been shown that N<sub>2</sub>O production under field conditions was a result of different microbial processes that were controlled by the interaction of many soil factors. Many crop management practices can affect N<sub>2</sub>O emissions either directly by affecting mineral N availability, or indirectly by modifying the soil microclimate and cycling of C and N (Snyder et al., 2009). Therefore, it is necessary to understand how management practices influence N<sub>2</sub>O emissions in order to develop strategies to mitigate environmental issues.



Numerous options for the mitigation of N<sub>2</sub>O emissions have been proposed, and they focus on increasing the efficiency of N fertilizer use and on reducing the amount of N cycling through an agricultural system (Saggar et al., 2008). Snyder et al. (2009) reviewed many management technologies and concluded that best management practices (BMPs) for fertilizer N play a large role in minimizing residual soil NO<sub>3</sub><sup>-</sup>, which helps lower the risk of increased N<sub>2</sub>O emissions. Such BMPs are soil management (no tillage and conservation tillage), N application management (N source, rate, timing, and placement), use of inhibitor and enhanced-efficiency sources, intensification of cropping systems, use of cover crops, etc. (Snyder et al., 2009).

Considering that NO<sub>3</sub><sup>-</sup> can accumulate in soils when the N rate exceeds crop demand and/or when crop recovery of the applied N is low, the primary consideration of applying the optimum N rate should be to minimize the surplus mineral N in soil solution without affecting economic return from grain yield (Van Groenigen et al., 2010). Recent field experiments suggest that significant decreases in N<sub>2</sub>O emissions may be possible by decreasing N fertilizer inputs (Hoben et al., 2011). In a study evaluating the effects of N rate (varying from 0 to 225 Kg N ha<sup>-1</sup>) on N<sub>2</sub>O flux, Hoben et al. (2011) found that N<sub>2</sub>O increased with increasing N application, especially at N rates greater than those required for maximum crop yield. In addition, the authors reported that at the two N fertilizer rates greater than the rate recommended for maximum economic return (135 kg N ha<sup>-1</sup>), the average N<sub>2</sub>O fluxes were 43% and 115% greater than were fluxes at the recommended rate.

The incorporation of CCs into cropping systems has been proposed as a best management practice to reduce N<sub>2</sub>O emissions from agricultural soils. Non-legume CCs have been found to be very effective in taking up mineral N from soil during the intercrop period, particularly cereals

(Kramberger et al., 2009). Cereal CCs belonging to the C<sub>4</sub><sup>2</sup> plant group can produce large amounts of biomass in a short growth period and can have the ability to capture high amounts of unused N after harvest that otherwise would be readily available for denitrification (Baligar and Fageria, 2007; Scharf, 2015). Although the cover crop N uptake is highly variable from one region to another (due to different soil and climate conditions), Tonitto et al. (2006) compared post-harvest N uptake by non-legume CCs and found an average of 37 Kg N ha<sup>-1</sup>, suggesting that CCs can scavenge a significant proportion of the excess mineral N after cash crop harvest. On the other hand, legume CCs can indirectly reduce N<sub>2</sub>O emissions by reducing N fertilizer requirements for the following cash crop, as they are able to symbiotically fix atmospheric N<sub>2</sub> and subsequently release some of that N after senescence and decomposition of the plant residues (Blanco-Canqui et al., 2015). In a review to assess the benefits of legume CCs on nitrogen management, Fageria et al. (2005) found that the quantity of N fixed varied from 24 to 177 Kg ha<sup>-1</sup> (depending on species and environment conditions), and the N fertilizer equivalence to succeeding cereal crops was on average, 77 and 34 kg ha<sup>-1</sup> after hairy vetch (*Vicia villosa* Roth) and crimson clover (*Trifolium incarnatum* L.), respectively. In a similar study, Sweeny and Moyer (2004) reported that the estimated N fertilizer equivalence was about 135 kg ha<sup>-1</sup> during the first year for grain sorghum following both hairy vetch and red clover (*Trifolium pretense* L.), suggesting that N fertilizer rates could be reduced accordingly.

In summary, mitigation strategies to reduce N<sub>2</sub>O emissions from agricultural soils can be achieved upon the understanding of the interactions among the factors that govern denitrification

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<sup>2</sup> Crop plants are classified as C<sub>3</sub> and C<sub>4</sub> based on the pathways of carbon metabolism and their behavior in CO<sub>2</sub> uptake (photorespiration). In general, the C<sub>4</sub> plants are better adapted to adverse environmental conditions compared to C<sub>3</sub> plants, and this may lead to a higher photosynthesis rate and consequently higher yield capacity (Fageria, 2009, pp. 15).

and how specific crop management practices affect emission rates. For this to happen, farmers should consider agronomic practices that provide the best trade-offs between GHG emissions and crop yield for a specific soil and weather condition.

### **Role of cover crops in cropping systems**

Worldwide, the sustainable use and quality of natural resources, especially soil and water, has become a priority for sustaining food production. Major practices used in sustainable agriculture production include crop rotations, reduced tillage, use of animal manures, and cover crops (Lu et al., 2000). Cover crops are an important component of a sustainable food production system because of their recognized ability to improve soil health, reduce environmental pollution, and improve crop yields (Fageria et al., 2005; Lu et al., 2000).

According to the Natural Resources Conservation Service of the U.S. Department of Agriculture, “CCs are defined as crops including legumes, grasses and forbs planted for seasonal vegetative cover and conservation purposes”. Moreover, CCs have the potential to provide multiple benefits in a cropping system, such as: preventing erosion, improving physical and biological properties of soil, supplying nutrients, suppressing weeds, improving soil water storage, breaking pest cycles, etc. (USDA-NRCS, 2014). It is important to highlight that these benefits vary by cover crop species, location, and season, but at least two or three usually occur with any cover crop (Clark, 2007).

Although growing CCs is an old practice, its role in agriculture has changed over time (Lu et al., 2000). In the past, CCs have been used for a few specific purposes (i.e., N supply, soil conservation, weed and pest management), but recently, the use of CCs in cropping systems revolve around its potential multi-functionality including benefits to soil health, mitigation of GHG, soil C sequestration, farm economics, and others (Blanco-Canqui et al., 2015). In fact, CCs

have been successfully incorporated into extensive cropping systems, including diverse cash grain rotations of Northern Europe, wheat-hay rotations in arid regions of Australia, and low-input grain systems in Africa (Tonitto et al., 2006).

Legumes, cereals, grasses, and brassicas have been used as CCs (Baligar and Fageria, 2007). Although CCs can be planted during all seasons of the year, they are usually planted after the cash crop is harvested (i.e., during the fallow period), therefore, they are referred to as winter and summer CCs (Clark, 2007). Hence, the winter CCs complement a summer cash crop, while the summer CCs complement a winter or fall cash crop (Snapp et al., 2005). For instance, both legume and cereal winter CCs such as hairy vetch, crimson clover, cereal rye, and barley, have been successfully managed in grain cropping systems in temperate regions (Tonitto et al., 2006), whereas several summer CCs have been used before fall vegetables in North Carolina (Creamer and Baldwin, 2000). In addition, winter legume cover crops have been proposed to improve yield.

Historically, legume CCs have been used as a source of N to the following cash crop while grasses have mainly been used to reduce erosion (Fageria et al., 2005). However, choosing the appropriate cover crop depends largely on the objectives of a farmer, whether to prevent soil erosion, reduce N loss, increase OM, as a source of N, or some other goal (Snapp et al., 2005). In addition, farmers also should take into consideration the cover crop growing cycle, biomass productivity, availability of commercial seeds, adaptability to a specific region, and tolerance of drought, heat, or cold (Borges et al., 2014).

### ***Benefits of cover crops***

Recently, CCs have received increased attention for their ability to enhance the multi-functionality of cropping systems, particularly in no-till farming (Blanco-Canqui et al., 2011). Growing CCs in rotation with cash crops in no-till cropping systems have the advantages of

reducing soil erosion (Blanco-Canqui et al., 2011; Blanco-Canqui et al., 2013), suppressing weeds (Hoffman and Regnier, 2006), improving soil physical properties (Blanco-Canqui et al., 2011; Chen and Weil, 2010), improving nutrient cycling (Boer et al., 2007), increasing farm income (Snapp et al., 2005), supplying N (Restovich et al., 2012; Schomberg et al., 2007; Vyn et al., 2000), reducing  $\text{NO}_3^-$  leaching and improving water quality (Dabney et al., 2001; Plaza-Bonilla et al., 2015; Strock et al., 2004), increasing crop yields (Mahama et al., 2015; Reinbott et al., 2004; Sweeney and Moyer, 2004), reducing diseases and insects (Bauer and Roof, 2004), and reducing GHG emissions (Sanz-Cobena et al., 2014). For this literature review, the effects of CCs on N management and crop yield are the main focus and will be more extensively discussed.

### ***Nitrogen management***

The benefit of legume CCs in cropping systems have long been recognized and has been attributed mainly to the N contribution to subsequent crops (Sarrantonio and Gallandt, 2003). Both legume and non-legume CCs can affect N fertilizer management (Bauer and Roof, 2004). For example, legume CCs can symbiotically fix atmospheric  $\text{N}_2$  and supply significant amounts of N in low-fertility soils after its decomposition, thereby supplementing N for the next crop and reducing N fertilizer requirements (Blanco-Canqui et al., 2015). On the other hand, non-legume CCs that produce biomass with a high C:N ratio may favor N immobilization and reduce available mineral N for the next crop, thereby increasing N fertilizer requirements. However, the N response of cash crops varies after different types of cover crops, and it is hard to predict with any certainty the quantity and timing of N availability following CCs (Sarrantonio and Gallandt, 2003).

The N content of the legume cover crop and the contribution of N to the following cash crop are strongly influenced by environmental and management factors (Reeves, 1994). These factors include soil moisture, temperature, and pH, which in turn are affected by weather, soil type,

tillage practices, and residue size and composition (Sarrantonio and Gallandt, 2003). Obviously, crop management practices that promote early establishment and development of the cover crop will result in greater biomass production and N concentration (Reeves, 1994). Hence, knowing the appropriate seed density, planting and termination dates, and methods of termination, would help farmers to better manage CCs between summer and winter cash crops (Schomberg et al., 2007).

A summary of recent studies relating biomass production and N uptake by different cover crops species, regions, and soil conditions are shown in Table 1.1. It was observed that under favorable conditions, the annual biomass production by legume CCs generally range from 1 to more than 10 Mg ha<sup>-1</sup>, and the N uptake capacity varies from 30 to more than 200 Kg ha<sup>-1</sup>. In contrast, non-legume CCs such as pearl millet and sorghum-sudangrass, generally produce higher amounts of biomass but with low N concentration. These values provide indication of two key aspects: (i) the potential capacity of a cover crop to uptake N from residual soil mineral N and mineralized soil OM as well as symbiotically fix N in the case of legume CCs; and (ii) that legume CCs have the capacity to accumulate more N in plant tissues than non-legume CCs (Fageria et al., 2005).

In addition to N fixation and subsequent supply, CCs have been used effectively to catch excess soluble N in the soil profile, reducing the potential for NL from agricultural lands (Sarrantonio and Gallandt, 2003). The CCs act as an “on-site method” to trap and store N during the intercrop periods. The CCs take up inorganic soil N and retain it in organic form in plant tissues, and then release the accumulated N to the next crop as the cover crop residue decomposes (Dinnes et al., 2002). However, the reduction in N loss varies from one region to another, weather and soil condition, and was dependent on the period that the cover crop was actively growing in the field (Robertson and Vitousek, 2009). In a meta-analysis of data using 14 studies, including

sites in United States, Sweden, Denmark, France, and Canada, Tonitto et al. (2006) found that NL was clearly reduced when a cover crop was present. In addition, the meta-analysis showed a 70% overall reduction in NL under the non-legume cover crop relative to bare fallow systems. Strock et al. (2004) found that adding cereal rye as a cover crop in a conventional corn-soybean rotation reduced NL by 13%. Under irrigated corn, Salmerón et al. (2010) found that barley and winter rape (*Brassica rapa* L.) reduced NL by 80% compared to continuous corn.

### ***Improving crop yield***

Increased yield of a marketable crop has been another major benefit that can be derived from growing CCs (Snapp et al., 2005), and was often a direct result of increased soil productivity over time (Reeves, 1994). Even though, increases in crop yield may vary from crop-to-crop, from region-to-region, and from year-to-year. In some cases, the amount of N provided by legume CCs is adequate to produce optimal yields of subsequent cereal crops (Fageria et al., 2005). On prairie soils in Kansas, for instance, Sweeney and Moyer (2004) found that grain sorghum following hairy vetch and red clover yielded approximately 130% more than continuous sorghum.

Several other studies have found significant yield responses of cereal crops following legume CCs when compared to bare fallow or cereal-cereal rotation. In Alabama, Balkcom and Reeves (2005) found that corn grain yield following sunn hemp (*Crotalaria juncea* L.) averaged 6.9 Mg ha<sup>-1</sup>, whereas, yield following winter fallow averaged 5.7 Mg ha<sup>-1</sup> (21% lower). In the southern Corn Belt in Missouri, Reinbott et al. (2004) found a significant increase in corn (8%) and grain sorghum yield (11%) after Austrian winter pea (*Pisum arvense*) and hairy vetch than after chemical fallow. Blanco-Canqui et al. (2012) found similar trends in a winter wheat-grain sorghum rotation. The authors reported that both sunn hemp and late maturing soybeans increased wheat yield by 70% in the first year of the rotation. However, the response in grain yield may not

be entirely due to the amount of available soil N (Fageria et al., 2005). Improvement in soil structure, fertility, and pest and weed control, have all been implicated in the yield response (Reeves, 1994).

The literature has shown the importance of incorporating legume CCs into cropping systems because of the increase in soil productivity and consequent increase in crop yields. However, the full benefit of using CCs will be dependent on the synchrony between cover crop N mineralization and N demand of the subsequent crop as well as an accurate estimation of supplemental fertilizer N requirements of the subsequent crop (Vyn et al., 2000). Moreover, conditions that increase the yield potential of the cash crop will increase the response to N (Reeves, 1994). Therefore, region- and site-specific research are needed to evaluate which cover crop species adapt better to the respective region and cropping systems and to adjust N fertilizer rates applied to major cash crops after different types of cover crops. This information would be of major importance to help producers to better manage and harvest the benefits of using CCs in their crop rotation.

### **Nitrogen use efficiency in crop production**

During the past 40 years, cereal yields and fertilizer N consumption have increased in a near-linear fashion and are highly correlated with one another (Dobermann and Cassman, 2005). In intensive agriculture regions, the use of large amounts of N fertilizer is often required to sustain/increase crop yields, but it also results in increased loss of N and therefore to deleterious effects on the environment (Lemaire et al., 2004). Therefore, the key to optimize tradeoffs between yield and environmental protection in most agriculture areas can be achieved by improving the synchrony between N supply and crop demand, without excess or deficiency (Cassman et al., 2002). In this sense, the NUE of crops has obtained great relevance, and it has several opportunities



for agricultural scientists to develop BMPs that increase the utilization efficiency of applied fertilizers (Fageria et al., 2008).

The determination of NUE in crop plants continues to be an important approach to evaluate the fate of applied chemical fertilizers and role in improved crop yields (Fageria and Baligar, 2005). However, one difficulty that arises has been that crops respond differently to N fertilizer, and the various methods used for calculating NUE makes it difficult to compare results of different studies (Fageria et al., 2008; Stewart et al., 2005). Furthermore, the effort to measure yield response is further confounded by other factors such as variable soil fertility levels, climatic conditions, crop rotations that affect NUE (Stewart et al., 2005). Nevertheless, it has been possible to make meaningful estimates of the contribution of N fertilizer inputs to crop yield and consequently NUE (Stewart et al., 2005).

### ***Definition and estimations of nitrogen use efficiency in plants***

Nitrogen use efficiency in plants has been defined in several ways (Fageria et al., 2008). Although, most of them denote the ability of a system to convert inputs into outputs (Fageria and Baligar, 2005). In general, NUE can be defined as the maximum economic yield produced per unit of N applied, absorbed, or utilized by the plant to produce grain and straw (Fageria and Baligar, 2005).

To measure NUE, the term most widely used has been a ratio that considers an output (biological or economic yield) as the numerator and an input (N supply) as the denominator (Ladha et al., 2005). For example, a common way to evaluate NUE in grain production has been based on the grain yield produced per unit of available N in the soil, as proposed by Moll et al. (1982). In this approach, the authors divided NUE into two primary components: (i) the efficiency of absorption (N uptake efficiency) and (ii) the efficiency with which the N absorbed was utilized to

produce grain (N utilization efficiency). Moll et al. (1982) also suggested the concept of N harvest index (NHI), which continues to be an important index to measure the re-translocation efficiency of absorbed N from vegetative plant parts to grain (Fageria, 2014). In many breeding programs where the crops are bred with focus on the food industry, the NHI has been of major importance to assess the grain quality of cereals. In a review study to evaluate NUE in crop plants, Fageria and Baligar (2005) reported a different approach to calculate NUE, and they grouped or classified the NUE as agronomic efficiency, physiological efficiency, and agro-physiological efficiency (Table 1.2).

There are two ways that are commonly used to calculate NUE and its components. The “N-difference” method is based on the difference in N uptake between a crop that receives a given amount of applied N and N uptake in a reference plot without applied N (Cassman et al., 2002). This approach can also be very useful for understanding N-cycling processes in agricultural systems because it allows one to differentiate N added by fertilizer from N already present in the system (Robertson and Vitousek, 2009). Another approach would be to use  $^{15}\text{N}$ -labeled fertilizer to estimate crop recovery of applied N (Cassman et al., 2002). The main challenge for NUE to be represented fairly in this method has been that the N added in form of fertilizer must represent the main source of N entering the agricultural systems, and the amount of organic N in soil has to be near steady state (Robertson and Vitousek, 2009).

Although fertilizer N consumption has increased in a near-linear fashion during the past 40 years, large differences exist in historical trends of N fertilizer usage and NUE of cereal (including wheat, corn, sorghum, rice, oat, rye, barley, and other minor cereal crops) among regions, countries, and crops (Table 1.3) (Dobermann and Cassman, 2005). Because NUE is a ratio, its value decreases with increased N application rates. This explains the high NUE value for Africa

(123 kg grain kg<sup>-1</sup> N) and Eastern Europe/Central Asia (90 kg grain kg<sup>-1</sup> N). In addition, differences in the average cereal NUE also depend on which crops are grown, their yield potential, soil and weather conditions, and amount and form of N applied (Dobermann and Cassman, 2005). Worldwide, the NUE of cereal crops was estimated to be approximately 33%, far less than the 50% generally reported (Raun and Johnson, 1999). But when taking into consideration the NUE in developed and developing countries, it averages only about 42 and 29%, respectively (Raun and Johnson, 1999). The remaining percentage of the N that is not absorbed by the plant either remains in the soil, which the recovery in the following crops is very limited, or it is lost from the soil-plant system. (Ladha et al., 2005).

Several studies have reported NUE in terms of N fertilizer recovery efficiency (RE<sub>N</sub>). Cassman et al. (2002) reported the average RE<sub>N</sub> of 37% for corn in the major corn-producing states of the United States. In addition, the authors reported that average RE<sub>N</sub> in lowland rice in the range of 31 to 40% in major rice growing regions in Asia. Fageria and Baligar (2001) reported that RE<sub>N</sub> in lowland rice in Brazil was 39% across 3 years. In India, the RE<sub>N</sub> averaged 18 and 49% for wheat grown under poor and good weather conditions, respectively (Cassman et al., 2002). In China, Zhu and Chen (2002) estimated that the mean RE<sub>N</sub> in rice, wheat, or barley, ranges from 30 to 35%, assuming a lower recovery in the high-yielding regions.

### ***Improving nitrogen use efficiency***

The above data illustrate that room remains to improve NUE at the farm level. The low recovery efficiency of N by most crop plants has been attributed to losses through volatilization, leaching, denitrification, and soil erosion (Fageria, 2014). Therefore, the overall NUE can be improved by adopting fertilizer, soil, water, and crop management practices that will maximize crop N uptake, minimize N losses, and optimize soil mineral N supply (Ladha et al., 2005). Raun

and Johnson (1999) estimated that an increase in NUE of 20% would result in a savings of more than \$4.7 billion per year. More recently, Dobermann and Cassman (2005) estimated that the anticipated 38% increase in global cereal demand by 2025 would be met with an increase of 20% in NUE.

Many approaches for improving the NUE of high-productivity agricultural systems have been identified, and can be grouped into three main options for annual cropping systems: (i) intensification of cropping systems with cover crops and cereal-legume rotations to improve the plant community's ability to take up more available N; (ii) provide farmers with decision support tools such as simulation models, remote sensing, precision farming technology, and variable rates, that allow them to better predict crop N requirements and avoid under- and over-fertilization; and (iii) better manage the timing, placement, and source of fertilizer N (Robertson and Vitousek, 2009). The BMPs proposed by Snyder et al. (2009) to mitigate N<sub>2</sub>O emissions from agricultural lands can also be applied in order to improve NUE, because these practices focus on increase N fertilizer use during the growing season by main crops when the fertilizer is applied as well as to decrease fertilizer N losses, thereby increasing the potential recovery of residual N by the subsequent crops (Ladha et al., 2005). Genetic tools are also of major importance for future improvement of NUE in crop plants. In fact, this strategy should continue to receive top priority for developing nutrient efficient crop genotypes to obtain higher yields in low input agricultural systems (Fageria et al., 2008).

### **Project hypothesis and objectives**

The hypotheses of this research were that: (i) including legume CCs in the fallow period after wheat harvest would increase N availability during the sorghum phase, while non-legume CCs would have similar or negative effects compared to the chemical fallow control, and this

would have a significant effect on the NUE by sorghum plants; (ii) all CCs would reduce N<sub>2</sub>O emissions during the fallow period and the legacy effects of CCs would reduce cumulative N<sub>2</sub>O emissions regardless of the N fertilizer rate applied during the sorghum cash crop phase.

The objectives of this study were to (i) determine the long-term effects of CCs and varying N rates on subsequent sorghum grain yield and yield components, (ii) assess how CCs affect the N dynamic in soil-crop relationship during the growing season and the consequent effect on NUE of sorghum plants, and (iii) define important periods of N<sub>2</sub>O losses and evaluate how different fallow management and N fertilization affects N<sub>2</sub>O emissions throughout the cropping systems.

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**Table 1.1.** Total biomass production and nitrogen (N) uptake by cover crops grown in different environments and soil conditions.

Study site	Soil	Cover crop	Biomass (Mg ha <sup>-1</sup> )	N uptake (kg ha <sup>-1</sup> )	years of experiment	Reference
Buenos Aires, Argentina	Silt loam	Rape seed ( <i>Brassica napus</i> L.)	1.1 - 6.1	18 - 172	5	(Restovich et al., 2012)
		Forage radish ( <i>Raphanus sativus</i> L.)	1.3 - 5.4	17 - 131	3	
Madrid, Spain	Silty clay loam	Hairy vetch	1.3 - 5.1	56 - 180	3	(Gabriel and Quemada, 2011)
		Barley	1.8 - 6.2	39 - 156		
Watkinsville, GA	Sandy loam	Sunn hemp	11.6	190	1	(Schomberg et al., 2007)
Tifton, GA	Sandy loam	Sunn hemp	10.4	198		
Shorter, AL	loamy sand	Sunn hemp	7.6	144	3	(Balkcom and Reeves, 2005)
Florence, SC	Loamy sand	Rye	0.68 - 2.50	5 - 17	3	(Bauer and Roof, 2004)
		Crimson clover	1.37 - 2.23	32 - 37		
Sao Paulo, Brazil	Sandy loam	Grain sorghum	13.9	23	2	(Borges et al., 2014)
		Pearl millet	7.9	19		
		Sorghum sudangrass	16.4	20		
Hutchinson, KS	Silt loam	Austrian winter pea	0.65 - 1.88	29 - 56	2	(Janke et al., 2002)
Ontario, Canada	Silt loam	Red clover	2.8 - 4.0	67 - 98	3	(Vyn et al., 2000)
		Forage radish	0.9 - 1.1	18 - 21		

**Table 1.2.** Definitions and methods of estimating the efficiency of nitrogen (N) use.

Nitrogen efficiency	Definitions and equations
N use efficiency (NUE)	Grain production per unit of available N in soil. Calculated by: $\text{NUE (kg kg}^{-1}\text{)} = \text{Gw}/\text{Ns}$ where Gw is the grain yield (kg ha <sup>-1</sup> ), and Ns is the N supplied (fertilizer N applied + N supplied in the soil) (kg ha <sup>-1</sup> ).
N uptake efficiency	Efficiency with which the plant uptake available N from the soil. Calculated by: $\text{NUpE (\%)} = (\text{Nt}/\text{Ns}) \times 100$ where Nt is the total aboveground N uptake (grain + straw) at maturity (kg ha <sup>-1</sup> ), and Ns is the N supplied.
N utilization efficiency	Efficiency with which the N absorbed is utilized to produce grain. Calculated by: $\text{NUE (kg kg}^{-1}\text{)} = \text{Gw}/\text{Nt}$ where Gw is the grain yield (kg ha <sup>-1</sup> ), and Nt is the total aboveground N uptake (grain + straw) at maturity (kg ha <sup>-1</sup> ).
N harvest index (NHI)	Efficiency of N partitioning in plant, i.e., how efficiently the plant re-translocate absorbed N from vegetative parts to grain. Calculated by: $\text{NHI (\%)} = (\text{Ng}/\text{Nt}) \times 100$ where Ng is the N accumulated in grain (kg ha <sup>-1</sup> ), and Nt is the total aboveground N in plant at maturity (kg ha <sup>-1</sup> ).
N recovery efficiency (RE <sub>N</sub> )	The quantity of N uptake per unit of N applied. Calculated by: $\text{RE}_N (\%) = (\text{Ntf} - \text{Ntu}/\text{Na}) \times 100$ where Ntf is the total N uptake of the fertilized plot (kg ha <sup>-1</sup> ), Ntu is the total N uptake of the unfertilized plot (kg ha <sup>-1</sup> ), and Na is the fertilizer N applied (kg ha <sup>-1</sup> ).
Agronomic efficiency (AE)	The economic production obtained per unit of nutrient applied. Calculated by: $\text{AE (kg kg}^{-1}\text{)} = \text{Gf} - \text{Gu}/\text{Na}$ where Gf is the grain yield of the fertilized plot (kg ha <sup>-1</sup> ), Gu is the grain yield of the unfertilized plot (kg ha <sup>-1</sup> ), and Na is the fertilizer N applied (kg ha <sup>-1</sup> ).
Physiological efficiency (PE)	The biological yield obtained per unit of nutrient uptake. Calculated by: $\text{PE (kg kg}^{-1}\text{)} = \text{DMf} - \text{DMu}/\text{Nf} - \text{Nu}$ where DMf is the dry matter yield (grain + straw) of the fertilized plot (kg ha <sup>-1</sup> ), DMu is the dry matter yield (grain + straw) of the unfertilized plot (kg ha <sup>-1</sup> ), Nf is the total aboveground N uptake of the fertilized plot (kg ha <sup>-1</sup> ), and Nu is the total aboveground N uptake of the unfertilized plot (kg ha <sup>-1</sup> ).
Agro-physiological efficiency (APE)	The economic grain yield obtained per unit of N uptake. Calculated by: $\text{APE (kg kg}^{-1}\text{)} = \text{Gf} - \text{Gu}/\text{Nf} - \text{Nu}$ where Gf is the grain yield of the fertilized plot (kg ha <sup>-1</sup> ), Gu is the grain yield of the unfertilized plot (kg ha <sup>-1</sup> ), Na is the fertilizer N applied (kg ha <sup>-1</sup> ), Nf is the total aboveground N uptake of the fertilized plot (kg ha <sup>-1</sup> ), and Nu is the total aboveground N uptake of the unfertilized plot (kg ha <sup>-1</sup> ).

Utilization efficiency (UE)      The product of physiological and N recovery efficiency. Calculated by:  
UE (kg kg<sup>-1</sup>) = PE x RE<sub>N</sub>.

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Source: compiled from Moll et al. (1982), Fageria et al. (2008), and Fageria and Baligar (2005).



**Table 1.3.** Cereal production, nitrogen (N) use on cereals, and N use efficiency (NUE) by world region (average for the 1999 to 2003 period).

	<b>Cereal production (MT)</b>	<b>N use on cereal (MT)<sup>a)</sup></b>	<b>N rate (kg ha<sup>-1</sup>)<sup>b)</sup></b>	<b>NUE (kg grain kg<sup>-1</sup> N)<sup>c)</sup></b>	<b>Relative NUE<sup>d)</sup></b>
<b><i>Developed regions</i></b>					
North America	377	12.5	112	45	1.0
NE Asia	19	0.9	89	71	1.6
W Europe	208	9.5	113	59	1.4
E Europe, C Asia	216	4.9	25	90	2.1
Oceania	34	1.3	48	46	1.1
<b><i>Developing regions</i></b>					
Africa	98	1.4	9	123	2.8
W Asia, NE Africa	81	4.2	68	34	0.8
South Asia	307	14.6	58	44	1.0
SE Asia	141	4.0	65	53	1.2
East Asia	447	24.9	155	32	0.7
Latin America	144	5.1	55	55	1.3
World	2072	83.2	70	44	1.0

Source: Dobermann and Cassman (2005).

a) Total fertilizer N consumption by all crops; b) Average estimated N application rate; c) Average NUE; d) Relative to world average (world = 1).

North America: USA and Canada; Northeast Asia: Japan and South Korea; Africa: Sub-Saharan and northwestern; West Asia and Northeast Asia: Near East, Turkey, Egypt and Libya; South Asia, Southeast Asia, and East Asia: China, Vietnam, and North Korea; Latin America: South and Central America, Caribbean).

## **Chapter 2 - No-till grain sorghum response to cover crop and nitrogen fertilization**

### **Abstract**

Nitrogen (N) is the most yield-limiting nutrient in sorghum [*Sorghum bicolor* (L.) Moench] production and cover crops (CCs) can affect N fertilizer management by influencing nutrient cycling and affecting N fertilizer requirement. A field study was conducted to (i) determine the long-term effect of CCs and N rates on subsequent sorghum growth and yield, and to (ii) assess how CCs affect the N dynamic in the soil-crop relationship during the growing season and the effect on N use efficiency (NUE) of sorghum plants. Plant tissue samples to determine total N uptake were collected after sorghum physiological maturity. Sorghum plots were harvested with a plot combine for grain yield determination. On average, double-crop soybean [*Glycine max* (L.) Merr] (DSB) and late-maturing soybean (LMS) provided one-third and one-half of the N required for optimum recommended rate, respectively, which resulted in increased grain yield when compared to other CCs and chemical fallow treatment (CF) with zero N application. Crimson clover (*Trifolium incarnatum* L.) and daikon radish (*Raphanus sativus* L.) had no or negative effects on sorghum yield and N uptake relative to CF across all N rates. Moreover, growing sorghum-sudangrass (*Sorghum bicolor* var. sudanese) prior to sorghum resulted in N immobilization and consequently reduced N uptake and grain yield even at higher N fertilizer rates. Sorghum following CF had the lowest NUE at maximum grain yield when compared to all CCs treatments, suggesting that CCs have a tendency to improve NUE. Overall, these results suggest that summer legume CCs or DSB have the potential to replace CF, and their strategic use can potentially decrease N fertilizer required to maximize sorghum yields, thus improving management of N resources in a wheat-sorghum cropping sequence.

Abbreviations:; 0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; ANM, apparent N mineralization; CCs, cover crops; DM, dry matter; NDVI, Normalized Difference Vegetation Index; NUE, nitrogen use efficiency.

## Introduction

Grain sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop produced in the world (FAOSTAT, 2015). Sorghum is an essential component of cropping systems in semi-arid tropical regions because of its tolerance of drought and high temperature. For this reason, sorghum is well adapted in most parts of Kansas, which allows it to be grown in regions with high summer temperatures and low precipitation, where other crops are more likely to fail or become unprofitable (Mahama et al., 2015). Kansas is the largest producer of grain sorghum in the United States, supplying 47% of the total production in the last 5 years (USDA-NASS, 2015). However, nitrogen (N) deficiency is still one of the major causes suppressing sorghum growth and grain production, particularly in developing countries.

The use of cover crops (CCs) have been long recognized as an important component of sustainable crop rotation, particularly in no-till farming (Lu et al., 2000). Long-term benefits of CCs are mainly attributed to their potential to improve soil health and reduce environmental pollution by improving nutrient cycling and reducing soil erosion (Fageria et al., 2005). Cover crops uptake nutrients before they leach below the rooting zone and release them later on or near the soil surface as residue decomposes, especially with deep-rooted species (Grove et al., 2007).

Legumes, cereals, grasses, and brassicas have been used as CCs (Baligar and Fageria, 2007). Both legume and non-legume CCs can affect N fertilizer management (Bauer and Roof, 2004). Legume CCs can symbiotically fix atmospheric N and supply significant amounts of N in low-fertility soils after its decomposition (Blanco-Canqui et al., 2015). On the other hand, non-

legume CCs that produce biomass with a high C:N ratio may favor N immobilization and reduce available mineral N for the next crop, thereby increasing N fertilizer requirements. However, the N content of the legume CCs and their contribution of N to the following cash crop are strongly influenced by several environmental and management factors (Reeves, 1994). Several long-term studies have assessed the benefits of legume CCs on nitrogen management. In a review study, Fageria et al. (2005) found that the quantity of N fixed varied from 24 to 177 kg ha<sup>-1</sup> (depending on cover crop species), and the N fertilizer equivalence to succeeding cereal crops were more typically between 50 to 100 kg ha<sup>-1</sup>. Researchers in the southeastern United States have shown that legume CCs such as hairy vetch (*Vicia villosa* Roth), crimson clover (*Trifolium incarnatum* L.), and sunn hemp (*Crotalaria juncea* L.) can supply 55 up to 130 kg N ha<sup>-1</sup> to following corn or grain sorghum crops (Balkcom and Reeves, 2005; Blevins et al., 1990; Oyer and Touchton, 1990).

In addition to N benefits, the increased yield of a marketable crop is another major benefit that can be derived from growing CCs (Snapp et al., 2005), and is often a direct result of improved soil quality and productivity over time (Reeves, 1994). Many studies have found significant yield responses of cereal crops following legume CCs when compared to chemical fallow or non-legume CCs. Sweeney and Moyer (2004) reported that grain sorghum following both hairy vetch and red clover (*Trifolium pretense* L.) yielded as much as 131% more than continuous sorghum. Reinbott et al. (2004) found a significant increase in corn (*Zea mays* L.) (9%) and sorghum (12%) yields after Austrian winter pea (*Pisum arvense*) and hairy vetch than after chemical fallow or oats (*Avena sativa* L.). Kramberger et al. (2009) and Restovich et al. (2012) found no yield advantage for corn following winter rape (*Brassica rapa* L.) or daikon radish (*Raphanus sativus* L.) when compared to chemical fallow.

Recent literature on improving nitrogen use efficiency (NUE) in crop-production systems has emphasized the need for greater synchrony between crop N demand and the N supply throughout the growing season (Cassman et al., 2002). Because the ability of CCs to both improve nutrient cycling and to supply and reduce N loss, many researchers have proposed that the intensification of cropping systems with CCs can improve NUE (Ladha et al., 2005; Robertson and Vitousek, 2009). However, if the increased soil N supply and/or the increased N uptake by plants is not associated with a significant increase in grain yield, it would not result in a positive effect on NUE. In addition, the dynamic nature of N in soil makes it more difficult to predict with any certainty the quantity and timing of N availability following CCs (Sarrantonio and Gallandt, 2003).

Although many studies have evaluated NUE response to varying N fertilizer rates, no study has assessed how long-term CCs can affect NUE in cereal grain crops such as sorghum. New research, measuring the N contribution of CCs in different agroecosystems will produce a better understanding of the impacts of CCs on NUE. This knowledge can further be used to develop N management strategies to maximize profit to farmers and to decrease N fertilizer requirements. Therefore, region- and site-specific research needs to both evaluate which cover crop species adapt better to the respective cropping systems and to adjust N fertilizer rates applied to major cash crops after different types of cover crops. This information will be of major importance to help producers to better manage and realize the benefits of using CCs in their crop rotation.

Thus, our hypotheses were that including legume CCs would increase N availability during the sorghum phase and consequently improve grain yield and N uptake, but non-legume CCs would have a negative or no effect compared to chemical fallow, and this would have a significant effect on the efficient use of N by sorghum plants. The objectives of this study were to (i) determine

the long-term effect of cover crops and N fertilizer rates on subsequent sorghum yield and N uptake, and (ii) to assess how CCs affect the N dynamic in soil-crop relationship during the growing season and the consequent effect on NUE of sorghum plants.

## **Material and methods**

### ***Site description and experimental design***

The study consisted of all phases of a three-year winter wheat (*Triticum aestivum* L.) – grain sorghum – soybean [*Glycine max* (L.) Merr] rotation (each component in the rotation was present every year). This study was established in 2007 as a long-term experiment at the Ashland Bottoms Research Farm of Kansas State University (K-State), located approximately 8 km south of Manhattan, KS (39° 11' N lat, 96° 35' W long). The altitude is approximately 311 m. The region has a humid subtropical climate (Köppen Climate Classification System: Cfa), with an average annual precipitation of 904 mm and annual mean temperature of 12.7°C (30-yr average). Precipitation and average monthly air temperatures in 2013, 2014, and 2015 are reported in Table 2.1. The soil was a moderately well drained Wymore silty clay loam (fine, smectitic, mesic Aquertic Argiudoll), with 0 to 1% slopes. Field measurements were conducted during the grain sorghum phase in the 2014 and 2015 growing seasons to evaluate the response of grain sorghum to CCs and varying N fertilizer rates. These treatments had been imposed three times (three full cycles of the rotation) since study inception in 2007. Mehlich-3 phosphorous (P) (Frank et al., 1998), exchangeable potassium (K) (Warncke and Brown, 1998), pH [1:1(v:v) soil:water mixture], and soil organic matter (SOM) (Ball, 1964) were measured before sorghum planting in each year (Table 2.2).

The experiment was arranged in a split-plot randomized complete block design with four replications. The cover crop treatments were the whole plots and N rates were the subplots. Each

whole plot was 6 by 70 m and each subplot was 6 by 14 m, comprising 8 sorghum rows. To minimize the border effects, all the plant samples were collected from the two center rows. The cover crop treatments included late-maturing soybean (LMS), sorghum-sudangrass (SS) (*Sorghum vulgare* var. sudanese), crimson clover (CL), daikon radish (DR), plus a chemical fallow (CF) treatment as a control and a double-crop soybean (DSB) treatment as a cash crop alternative following winter wheat. The N fertilizer rates consisted of a control (zero N) and four N rates (45, 90, 135, and 180 kg ha<sup>-1</sup>; hereafter 0-N, 45-N, 90-N, 135-N, and 180-N, respectively) that were applied as 28% Urea Ammonium Nitrate (UAN) immediately after sorghum planting.

### ***Winter wheat and cover crop management***

Winter wheat was planted immediately after soybean harvest in October 2012 and 2013 with a target seeding rate of 115 kg ha<sup>-1</sup> on 19-cm rows using a John Deere 1590 no-till drill (Deere & Co., Moline, IL). Diammonium phosphate was applied at planting with wheat seed at a rate of 65 kg ha<sup>-1</sup>, supplying ~12 and 30 kg of N and P ha<sup>-1</sup>, respectively. In March of 2013 and 2014, the wheat was top-dressed with 67 kg N ha<sup>-1</sup> soon after spring greenup using 28% UAN, applied in streams spaced every 10 cm. The weeds were controlled by applying glyphosate [N-(phosphonomethyl)glycine] at a rate of 1.67 kg a.i. ha<sup>-1</sup> after planting the wheat, and 21 g a.i. ha<sup>-1</sup> of thifensulfuron-methyl plus 10.5 g a.i. ha<sup>-1</sup> of tribenuron-methyl (Harmony® Extra SG, DuPont) after spring greenup. Winter wheat was harvested in 16 June 2013 and 2 July 2014.

The planting dates of CCs varied between years. In 2013, the summer CCs (LMS and SS) and DSB were planted immediately after wheat harvest on 17 June 2013, whereas the winter CCs (CL and DR) were planted on 3 Oct 2013. In 2014, all CCs and DSB were planted after wheat harvest on 3 July 2014. The target seeding rate was 23 kg ha<sup>-1</sup> for SS and CL, 12 kg ha<sup>-1</sup> for DR, and 420,000 seeds ha<sup>-1</sup> for LMS and DSB. After CCs and DSB planting, the plots were sprayed

with glyphosate at a rate of 1.67 kg a.i. ha<sup>-1</sup>. Prior to termination of CCs, aboveground biomass was sampled from a 1.16 m<sup>2</sup> area in the center of the plots to determine dry matter (DM) yield and N and carbon (C) content. Subsamples from each cover crop were oven-dried at 60°C for 7 days, weighed, ground to pass a 2-mm screen with a Wiley Mill (Thomas Scientific, Swedesboro, NJ), and analyzed for N and C by dry combustion using a LECO CHN-2000 elemental analyzer (LECO Corp., St. Joseph, MI, USA) at the K-State Soil Testing Laboratory. The remaining fresh plant material cut from the cover crop plots was then evenly distributed on the respective harvested area and left in the field to decompose. The summer CCs were terminated using a roller-crimper at beginning of flowering (approximately 10 weeks after planting). The winter CCs were killed by freezing temperatures, which usually occurred during November. The DSB plots were harvested with a plot combine on 20 Oct 2013 and 7 Nov 2014. In March of 2014 and 2015, all plots were sprayed with 1.46 kg a.i. ha<sup>-1</sup> of glyphosate plus 0.53 kg a.i. ha<sup>-1</sup> of 2,4-D (2,4-Dichlorophenoxyacetic acid) to kill volunteer wheat and other winter annual weeds.

### ***Grain sorghum management***

Grain sorghum was planted on 19 May 2014 and 1 June 2015, with a target seeding rate of 119,000 seeds ha<sup>-1</sup>. Planting was performed using a White 6200 4-row planter (AGCO Corp., Duluth, GA) to a depth of 2.5 to 3.5 cm and 76-cm row spacing in both years. A medium-maturity hybrid (Pioneer 85G03) and medium-late maturity hybrid (DKS 53-67) were planted in 2014 and 2015, respectively. Nitrogen fertilizer rates were subsurface banded on 4 June 2014 and 2 June 2015, using 28% UAN with a straight flat-coulter liquid fertilizer applicator to inject N fertilizer below the residue layer. No P or K fertilizer was applied. Sorghum was sprayed with 1.25 kg a.i. ha<sup>-1</sup> of glyphosate and a herbicide in the form of 2.26 kg a.i. ha<sup>-1</sup> of acetochlor [2-chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl)acetamide] and 1.12 kg a.i. ha<sup>-1</sup> of atrazine [2-chloro-4-



(ethylamino)-6-(isopropylamino)-s-triazine] (Degree XTRA®, Monsanto) within 2 days after planting to control post-emergence of weeds in both years. When needed, hand weeding was also done throughout the growing season to maintain plots free of weeds.

The canopy sensor GreenSeeker® (Trimble Navigation Ltd., Sunnyvale, CA) was used to determine in-season leaf N status of sorghum plants. Normalized Difference Vegetation Index (NDVI) was obtained twice during the growing season: at growth Stage 3 (growing point differentiation) and 5 (Boot stage) in both years. The data was collected from a 10 m row per plot (center row of each plot) and the sensor was held at a height of about 1 m above the plant canopy. In 2015, leaf chlorophyll index of the first leaf below the flag leaf was measured at growth Stage 5 from each plot using a chlorophyll meter (Model 502 PLUS, Konica Minolta, Tokyo, Japan.). Each plot reading consisted of the average of 20 plants from a 10 m row.

After sorghum physiological maturity, DM yield was determined by hand harvesting plant samples from 0.76 m<sup>2</sup> area in the center of each plot on 9 Sept 2014 and 28 Sept 2015. The plants were clipped close to ground level and separated into two components: panicles and stover (stems + leaves), and weighed in the field. Sorghum stover was then chopped to 2.5 cm length using a chipper shredder (Model CS 3310, Cub Cadet, Valley City, OH), mixed thoroughly, and a representative subsample of about 300 g was collected for determination of DM yield and N concentration. Both panicles and stover were dried at 60°C in a forced-air oven until constant weight. The dried stover was ground to pass a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). The panicles were threshed in a stationary thresher (Model SPVT, ALMACO, Nevada, IA) and the grain samples were ground to a fine powder in a coffee grinder (Model Rocky Doserless, Rancilio Group, Woodbridge, IL). The concentration of N in both stover and grain samples were determined using the salicylic-sulfuric acid digestion method (Bremner and

Mulvaney, 1982) at the K-State Soil Testing Laboratory. Based on the sampling area, total DM production was determined by the sum of panicle and stover weight and expressed per unit area ( $\text{kg ha}^{-1}$ ). Nitrogen accumulated in stover and in grain, and total N uptake in plant were calculated using the following equations:

$$\text{N accumulated in stover (kg ha}^{-1}\text{)} = \text{stover yield} \times (\text{N\% in stover}/100) \quad \text{Eq. [1]}$$

$$\text{N accumulated in grain (kg ha}^{-1}\text{)} = \text{grain yield} \times (\text{N\% in grain}/100) \quad \text{Eq. [2]}$$

$$\text{Total N uptake (kg ha}^{-1}\text{)} = \text{N accumulated in stover} + \text{N accumulated in grain} \quad \text{Eq. [3]}$$

Sorghum was harvested from the two center rows of each plot with a modified 2-row Gleaner Model E-II combine (AGCO Corp, Duluth, GA) for yield determination on 08 Oct 2014 and 15 Oct 2015. Grain moisture and test weight were measured with a moisture meter (Model GAC 2000, DICKEY-John Corp., Springfield, IL), and grain yields in both years were corrected to  $125 \text{ g kg}^{-1}$  moisture content.

### ***Components of nitrogen use calculations***

Nitrogen use efficiencies were calculated according to the following equations proposed by Moll et al. (1982), Dobermann (2007), and Fageria and Baligar (2005):

$$\text{N use efficiency (kg ha}^{-1}\text{)} = \text{Gw}/\text{Ns} \quad \text{Eq. [3]}$$

$$\text{N utilization efficiency (kg ha}^{-1}\text{)} = \text{Gw}/\text{Nt} \quad \text{Eq. [4]}$$

$$\text{N uptake efficiency (\%)} = (\text{Nt}/\text{Ns}) \times 100 \quad \text{Eq. [5]}$$

$$\text{N recovery efficiency (\%)} = [(\text{Ntf} - \text{Ntu})/\text{Na}] \times 100 \quad \text{Eq. [6]}$$

$$\text{N harvest index (\%)} = (\text{Ng}/\text{Nt}) \times 100 \quad \text{Eq. [7]}$$

$$\text{Partial Factor Productivity (kg kg}^{-1}\text{)} = \text{Gw}/\text{Na} \quad \text{Eq. [8]}$$

where Gw is the grain yield; Ns is the N supplied (fertilizer N applied + N supplied in the soil; N supplied by soil was estimated by total N in plant for 0-N plot); Nt is the total N uptake; Ntf is the

total N uptake of the fertilized plot; Ntu is the total N uptake of the unfertilized plot; Na is the N fertilizer applied; and Ng is the N accumulated in grain. All parameters used in the calculations had the same units of kg ha<sup>-1</sup>.

### ***Nitrogen fertilizer replacement value***

Linear and quadratic regression analysis were used to describe the relationship between sorghum grain yield and N fertilizer rate for each year. The effect of N fertilizer on grain yield was identified using linear and quadratic contrasts. The nitrogen fertilizer replacement value (NFRV) from CCs and DSB was calculated with a method similar to that used by (Oyer and Touchton, 1990). Briefly, fertilizer N replacement provided by each cover crop and DSB was estimated for each year by solving the quadratic equation after substituting the mean grain yield at the 0-N rate for each cover crop treatment into the N response equation for the CF treatment of the respective year.

### ***Statistical analysis***

Data were analyzed using a split-plot randomized complete block design, with cover crop treatments as the whole plot factor and N rate as the subplot factor. The effects of CCs, N rates, and their interaction were evaluated by ANOVA. Fisher's Least Significant Difference method was used to assess the difference between the means of the treatments. The ANOVA and mean separation differences were carried out using PROC GLIMMIX of the SAS<sup>®</sup> software (SAS Institute, 2011). Year was included in the analysis and treated as a fixed effect to determine interactions involving year. The ANOVA for the cover crop measurements was conducted by year, due to missing values in the 2013 data. Main effects and all interactions were considered significantly different at  $P \leq 0.05$ . In addition, to evaluate the degree of association between the

response variables, the data were submitted to analysis of correlation and regression using PROC CORR and PROC REG.

## **Results**

### ***Weather condition and soil analysis***

The precipitation during the growth of CCs and double-crop soybeans (July – October) after wheat harvest varied between years, as shown in Table 2.1. Although total precipitation between July and October was greater in 2013 (275 mm) compared to 2014 (215 mm), only 30% of the rainfall occurred in the first two months after CCs and double-crop soybean planting. The maximum temperature in June was 1.2°C above the 30-yr normal in 2015. Precipitation amounts during the grain sorghum growing season also varied between years, with greater precipitations in 2015 (436 mm) than 2014 (368 mm) (Fig 2.1). In general, rainfall events were more evenly distributed throughout the 2015 growing season.

The results of the soil analysis indicated K was above the critical level of 160 mg kg<sup>-1</sup> in both years (Table 2.2). Mehlich-3 P was also above the critical level of 20 mg kg<sup>-1</sup> in 2014, but it was 1 mg kg<sup>-1</sup> below in 2015. Higher SOM content was observed in 2015 than the previous year.

### ***Cover crop biomass and nitrogen production***

Total DM production, N content, N concentration, and C:N ratio were all significantly affected by the type of cover crop in both years ( $P \leq 0.05$ ), but they were not significantly affected by N rate or the cover crop by N interaction. In 2013, the winter CCs were not sampled prior to termination, and consequently, those treatments were eliminated from the analysis for that year and reported as missing values.

In both years, SS produced the greatest amount of DM when compared to the other CCs (Table 2.3). In 2014, LMS produced twice as much DM when compared to 2013. In 2014, the DM

production of CL was 3.3 Mg ha<sup>-1</sup> compared to 2.7 Mg ha<sup>-1</sup> of DR, although the difference was not significant.

The N concentration of CCs followed a similar trend in both years, and it was strongly correlated with C:N ratio ( $R^2 = 0.9917$ ;  $P < 0.0001$ ). In 2014, LMS had the greatest N concentration, followed by CL, DR, and SS. The C:N ratio was lowest for LMS (16:1) but not significantly different from CL (18:1). In both years, SS had the greatest C:N ratio of 39:1.

The N content of CCs varied by year, and it was significantly correlated with the N concentration and DM production ( $R^2 = 0.7557$ ;  $P < 0.001$ ). In 2013, SS had the greatest N content when compared to LMS. In 2014, LMS accumulated more N in plant tissues (99.3 kg ha<sup>-1</sup>) compared to other CCs. In addition, there was no significant difference in N content between SS (75.5 kg ha<sup>-1</sup>) and CL (79.5 kg ha<sup>-1</sup>). Daikon radish had the lowest N content in 2014 (42.3 kg ha<sup>-1</sup>).

### ***In-season leaf nitrogen status of sorghum***

The 3-way interaction of cover crop  $\times$  N rate  $\times$  year was significant for NDVI at Stage 3 ( $P = 0.001$ ), but not for Stage 5 ( $P = 0.273$ ) (Table 2.4). Therefore, the interaction of cover crop by N rate was reported separately for each year for NDVI at stage 3, and pooled with years for NDVI at stage 5.

In general, the spectral behavior of sorghum at growth stage 3 varied by year (Fig 2.2a-b). In 2014, the use of a SS cover crop prior to grain sorghum significantly reduced NDVI across all N rates when compared with the other cover crop treatments (Fig 2.2a). The NDVI readings saturated at 45-N for sorghum grown after DSB and LMS, at 135-N after CF and CL, and at 180-N after DR. In addition, where no N fertilizer was applied, NDVI was higher on sorghum planted after DSB when compared to CF and CL, but it was not different after LMS and DR. In 2015, there

was no significant difference in NDVI across N rates for all cover crop treatments, except SS (Fig 2.2b).

When averaged across years, a similar trend was observed at growth stage 5, where the reduced NDVI readings persisted in sorghum planted after SS across all N rates (Fig 2.3a). The NDVI readings saturated at 45-N after CF and CL, and they were not significantly different at higher N rates. No difference was observed in NDVI values across all N rates for sorghum grown after DSB, LMS, and DR. When averaged across N rates, the effect of CCs on NDVI at growth stage 5 was similar in both years (Fig 2.3b). No significant difference was observed on NDVI for all cover crop treatments in each year, except SS, which reduced NDVI value by 0.1 in 2014.

The relationship between NDVI and sorghum grain yield was highly significant ( $P < 0.0001$ ) at both growth stages in both years (Fig 2.4). In 2014 and 2015, the coefficient of determination ( $R^2$ ) was less at growth stage 5 (Fig 2.4b and d) than at growth stage 3 (Fig 2.4a and c). Similar to grain yield, a quadratic regression model showed that there was a significant relationship between total N uptake and NDVI (Fig 2.5). However, the  $R^2$  was higher in 2014 (0.56 and 0.58 for growth stage 3 and 5, respectively) than in 2015 (0.35 and 0.33 for growth stage 3 and 5, respectively).

Analysis of variance showed a significant interaction between cover crop and N rate on leaf chlorophyll index at growth stage 5 (Table 2.4). In general, the leaf chlorophyll index increased with increasing N rates in all cover crop treatments, except for the CF and DSB (Fig. 2.6). For these two treatments, leaf chlorophyll index followed a quadratic trend. However, no significant difference was observed among the four N fertilizer rates, except for the SS treatment. Where no fertilizer was applied, sorghum planted after SS had the lowest leaf chlorophyll index, whereas there was no significant difference among the other CCs and CF. The regression models

between leaf chlorophyll index and total N uptake (Fig 2.7a), and grain yield (Fig 2.7b) showed a significant relationship, with  $R^2$  values of 0.70 and 0.74, respectively.

### ***Sorghum grain yield, dry matter, and nitrogen uptake***

The three-way interaction of cover crop  $\times$  N rate  $\times$  year was not significant for grain yield ( $P = 0.47$ ), DM ( $P = 0.83$ ), and total N uptake ( $P = 0.59$ ), as shown in Table 2.5. The interaction between cover crop and N rate was significant for grain yield and DM but not for N uptake; therefore, the data were pooled across both years.

In general, the response of sorghum grain yield to N rates was similar for most cover crop treatments. Grain yield was greatest when N was applied at a rate of 135 kg ha<sup>-1</sup> for all cover crop treatments except SS and DSB (Fig 2.8). For sorghum grown after these two latter cover crop treatments, grain yield was greater at 180-N (Fig 2.8b and d). However, no significant difference was observed in grain yield when N rate increased from 135-N to 180-N for SS, and from 90-N to 180-N for the remaining cover crop treatments. Where no fertilizer was applied, grain yield was significantly more in sorghum planted after LMS (6.59 Mg ha<sup>-1</sup>) when compared to CF (5.96 Mg ha<sup>-1</sup>) and the other CCs, but it was equivalent to that after DSB (6.29 Mg ha<sup>-1</sup>). Overall, grain yield of sorghum planted after LMS with 0-N was 10% greater than after CF with 0-N, and it was greater and/or equivalent to that after all CCs and DSB at 45-N. The 95% maximum economic yield occurred at 135-N for sorghum planted after SS and DR, and at 90-N for the remaining treatments.

The regression models relating grain yield to total N supplied (Fig 2.9a) and total N uptake (Fig 2.9b) showed a highly significant ( $P < 0.0001$ ) quadratic relationship in both years. Based on the regression lines, maximum grain yield peaked around 200 and 275 kg of N supplied ha<sup>-1</sup> in 2014 and 2015, respectively. A similar trend was observed when grain yield was expressed as a function of total N uptake.

Because DM production was strongly correlated with grain yield ( $R^2 = 0.8862$ ;  $P < 0.0001$ ), the DM response to CCs and N rates had a similar trend with that of grain yield (Fig 2.10a). Sorghum DM production significantly increased with increasing N rates when planted after SS. For all the remaining cover crop treatments, maximum DM occurred at 180-N and ranged from 17.3 to 18.1 Mg ha<sup>-1</sup>, however, they were not significantly different when N rates were reduced to 90-N. Overall, sorghum planted after SS with 180-N (16.1 Mg ha<sup>-1</sup>) produced about the same amount of DM as sorghum following LMS (15.7 Mg ha<sup>-1</sup>) and DSB (14.9 Mg ha<sup>-1</sup>) with 0-N. In 2014, increasing N rate from 90-N to 180-N did not increase DM production, but it did increase in 2015 (Fig 2.10b).

For total N uptake, only the interaction between cover crop and year and the main effects were significant (Table 2.5). In general, the cover crop effects on N uptake were similar in both years (Fig 2.11a). Growing SS before sorghum resulted in a significant decrease in N uptake in both years. In 2014, LMS increased N uptake compared to CL and SS, but it was not statistically different from the other cover crop treatments and CF. In 2015, no difference was observed among most of the cover crop treatments and DSB, except DR and SS. When averaged across years and CCs, total N uptake significantly increased with increasing N fertilizer rate (Fig 2.11b). Total N uptake from the 0-N control treatment was used to estimate the apparent N mineralization (ANM) for each cover crop treatment. Overall, LMS significantly increased ANM when compared to other treatments, except DSB (Table 2.6). Sorghum-sudangrass had the least ANM.

### ***Components of nitrogen use efficiency***

The 3-way interaction of cover crop  $\times$  N rate  $\times$  year was not significant for N use efficiency (NUE) and the components of N efficiency (Table 2.7). Moreover, Nitrogen fertilizer recovery



efficiency ( $N_{RE}$ ) was not significantly affected by any main effects and their interactions (Table 2.7).

There was a significant cover crop by year interaction for NUE, but the interaction between cover crop and N rate was not significant; therefore, the data was pooled with N rates. In general, the effects of CCs on NUE varied by year (Fig 2.12a). In general, NUE for all cover crop treatments were higher in 2015 than in 2014, except for CL. In 2014, CL increased NUE ( $38.5 \text{ kg kg}^{-1}$ ) when compared to DR ( $34.6 \text{ kg kg}^{-1}$ ) and LMS ( $31.4 \text{ kg kg}^{-1}$ ), but it was not significantly different from CF, DSB, and SS. In addition, LMS had the lowest NUE among the cover crop treatments in 2014. Values of NUE following most cover crop treatments and DSB were similar to those after CF in 2015, and they ranged from  $40.6 \text{ kg kg}^{-1}$  to  $42.5 \text{ kg kg}^{-1}$ . In both years, NUE significantly decreased with increasing N rate (Fig 2.12b).

The interactions and main effects were significant for N utilization efficiency ( $NUtE$ ) (Table 2.7). Increasing N rates resulted in a significant reduction of  $NUtE$  for all cover crop and control treatments, except SS (Fig 2.13a). Overall,  $NUtE$  was higher for SS across all N rates. Where no fertilizer was applied, no significant difference was observed among the cover crop treatments except for LMS, which had the lowest  $NUtE$ . At 90-N, CF significantly reduced  $NUtE$  by 14 and 17% when compared to CL and DR, respectively. At 180-N, SS significantly increased  $NUtE$ , while there was no difference among the other cover crop treatments. When averaged across N rates, SS significantly increased  $NUtE$  in both years (Fig 2.13b). In 2014, LMS had the lowest  $NUtE$ . In 2015, CF significantly reduced  $NUtE$  when compared to LMS, SS, and DR.

Cover crop, N rate, and year significantly affected N uptake efficiency ( $NUpE$ ) but with no significant interaction among main effects (Table 2.7). When averaged across N rates and years, sorghum planted after SS had the lowest  $NUpE$  when compared to all cover crop treatments, except

DR (Table 2.8). Across cover crop treatments and years, NUpE was higher at 0-N, and decreased with increasing N rate. Moreover, NUpE was higher in 2015 than in 2014

Effect of CCs was not significant for N harvest index (NHI), but its significant interaction with N rate was observed (Table 2.7). Increasing N rate resulted in a significant reduction of NHI on sorghum grown after CF and DSB (Fig 2.14a). No significant difference was observed on NHI between 90-N and 180-N for LMS, SS, CL, and DR. Overall, NHI ranged from 56.4 to 66.9%. When averaged across cover crop and year, NHI was higher at 0-N (64.6%) compared to 180-N (59.4%), but not statistically different from 90-N (63.4%) (Fig. 2.14b).

There was significant effect of the interactions and main effects on Partial Factor Productivity (PFP) (Table 2.7). In general, PFP significantly decreased with increasing N fertilizer rate for all cover crop treatments (Fig 2.15a). In addition, PFP started to equalize at 90-N (except for SS), and no difference was observed among the cover crop treatments at 135-N and 180-N. At 45-N, LMS had the highest PFP when compared to other CCs and DSB, but it was similar to CF. When averaged across N rates, the CCs had similar effects in both years (Fig 2.15b). Sorghum planted after SS had the lowest PFP in both years. In 2015, the PFP of sorghum following DR was significantly less than from the other cover crop treatments, whereas no difference was observed among the cover crop treatments in 2014, except SS.

### ***Nitrogen fertilizer replacement value***

Linear and quadratic regression equations for sorghum grain yield as a function of N fertilizer were determined for each year. The contrast tests indicated that grain yield followed a quadratic trend as a function of N fertilizer in both years (2014:  $P = 0.002$ ; 2015:  $P = 0.0002$ ). Based on these results, the quadratic regression improved the fit when compared to linear regression (Table 2.9); hence, it was used to estimate the NFRV for each cover crop.

Although substantial variability was seen between years, a similar trend was observed on the NFRV contributed by each cover crop and DSB (Table 2.10). In 2014, both LMS and DSB provided more than 52 kg N ha<sup>-1</sup> and CL provided up to 35 kg N ha<sup>-1</sup> to the subsequent cash crop in the rotation. On the other hand, DR and SS had little or negative effects on NFRV. In 2015, all cover crop treatments had negative NFRV, except LMS and DSB. When averaged across years, the mean estimated NFRV was greater for the LMS treatment, followed by DSB and CL. Both DR and SS resulted in negative NFRV.

## **Discussion**

### ***Cover crop biomass and nitrogen production***

The total aboveground dry matter production of legume CCs were greater in 2014-15 growing season than in the 2013-14 growing season. The pattern of rainfall distribution was different between years, while temperature patterns were similar (Table 2.1). It is well known that water is important for plant growth and development. Soil water content not only affect the amount of nutrient in the soil solution (by controlling microbial growth and activity), but the rate of movement to the root (by diffusion and mass flow) as water is absorbed by the plants, in which lesser amounts of water result in progressively poorer conditions for nutrient availability (Viets, 1972). Therefore, it is plausible that the extra precipitation that occurred in August of 2014 resulted in adequate soil moisture, which may have contributed to an increased nutrient availability and consequent early biomass accumulation. Regarding the LMS, these results indicate that when water is not a limiting factor, it has the potential to produce large amounts of residues (3.7 Mg ha<sup>-1</sup>) in a short period of about 10 weeks, accumulating up to 100 kg N ha<sup>-1</sup> (Table 2.3). Similar results were observed in south central Kansas by Blanco-Canqui et al. (2012). The authors found that

biomass production of LMS ranged from 2.5 to 3.2, and from 3.5 to 5.6 Mg ha<sup>-1</sup> within 10 and 12 weeks after planting, respectively.

Although SS tended to be more productive in terms of DM yield, it had the lowest N concentration, and therefore, the highest C:N ratio. When averaged across years, N concentration of legume CCs was 25.5 g kg<sup>-1</sup>, which was 1.9-fold greater than the non-legume CCs. The higher N concentration in legume CCs is the result of the symbiotic association with bacteria species (*Rhizobium* and *Bradyrhizobium*) that are responsible for atmospheric N fixation (Reeves, 1994). Our results were similar to those reported by Vyn et al. (2000) and Bauer and Roof (2004). The latter authors reported that CL grown in South Carolina produced about 1.78 Mg ha<sup>-1</sup> of DM, but with greater N concentration (37 g kg<sup>-1</sup>) when compared to our results. Vyn et al. (2000) reported that DR grown in Canada yielded only 0.95 Mg ha<sup>-1</sup> and accumulated 20 kg N ha<sup>-1</sup>. However, cover crop performance was very dependent on other factors such as weather, precipitation, soil type, planting and termination dates, etc.

### ***In-season nitrogen leaf status of sorghum***

Photosynthetic activity (which is associated with chlorophyll concentration) has been successfully predicted by NDVI because this vegetation index includes both near infrared and red light (Verhulst and Govaerts, 2010). Generally, greater leaf area and green plant biomass levels result in higher NDVI values, and because these variables are directly related to the N content of the plant, higher NDVI values indicate higher plant N content (Shaver et al., 2011).

The effects of CCs and N rate on NDVI readings at growth stage 3 was significantly different between years. Overall, more variability among treatments was observed in 2014 than in 2015. A plausible explanation is the different N management practices coupled with contrasting precipitation pattern between years (Fig 2.1). In 2014, N fertilizer was applied 15 days after

sorghum planting, and NDVI readings at growth stage 3 were taken 16 days after fertilizer application. During this latter period, sorghum received about 137 mm of precipitation. In 2015, N fertilizer was applied the day after sorghum planting, and received about 185 mm of precipitation that was evenly distributed until the first NDVI measurements. Vanderlip (1993) reported that soil temperature and nutrient and water stress are the main factors limiting sorghum growth before 20-25 days after emergence. In this sense, the higher rainfall coupled with higher nutrient availability (more N dissolved in soil solution readily available for plant uptake due to sooner N application) in 2015 (Tables 2.1 and Fig 2.1) may have resulted in better growing conditions early in the season for plant development, and consequently, reduced variability among treatments.

At growth stage 5, the NDVI readings had less variability among cover crop and N rate treatments. This may be due to the fact that 30 days after emergence sorghum plants have rapid growth and nutrient uptake (Vanderlip, 1993). At growth stage 5, the plant is about 1 meter tall, and all leaves are fully expanded, almost closing the canopy. In situations where the majority of the soil is covered by the plant canopy, there is a peak of absorption in the red light wavelength (Povh et al., 2008). When this happens, NDVI becomes less sensitive to change in biomass and consequently result in smaller differences in NDVI values. However, the NDVI was lower for sorghum grown after SS even at higher N rates. It is possible that the high DM and C:N ratio of SS may have favored N immobilization, and therefore, the significant lower NDVI readings were due to lower leaf N concentration and biomass when compared to other cover crop treatments (Figs 2.10 and 2.11).

NDVI values at growth stage 3 showed better correlation ( $R^2 = 0.58$ ) with final grain yield than at growth stage 5 ( $R^2 = 0.53$ ) in 2014. The  $R^2$  at both growth stages in our study were relatively low when compared to the literature, perhaps reflecting the high variability among replications

(with coefficient of variation up to 27%). Although few studies have defined relationships between NDVI and sorghum grain yield, most have shown different results. Tucker (2009) reported that the correlation between NDVI and yield increased from 21 days after planting ( $R^2 = 0.20$ ) to 35 days after planting ( $R^2 = 0.67$ ), then reduced and remained constant ( $R^2 = 0.62$ ) for the next 2 weeks. Moges et al. (2007) found a better correlation ( $R^2 = 0.75$ ) at growth stage 3 when data were combined over 2 years of an experiment in Oklahoma. Moreover, Rosales-Rodriguez (2014) found a  $R^2$  of 0.48 and 0.68 after 31 and 50 days after emergence, respectively for sorghum in Arkansas.

### ***Grain yield, dry matter, and nitrogen uptake***

When averaged across years, contrast tests showed a highly significant linear response of sorghum grain yield in relation to N fertilizer rate, and a quadratic response for the CF and DR treatments. In most cover crop treatments and DSB, increasing N fertilizer applications from 90-N to 180-N had no effect on sorghum grain yield, indicating that 90-N was sufficient to optimize yields (Fig 2.8). However, maximum grain yield for sorghum grown after DR occurred at 135-N. Sorghum planted after SS resulted in a significant linear response ( $P < 0.0001$ ) to N fertilizer rates, indicating that available N was limited even after applying 135-N. The reduction in grain yield, especially at lower N fertilizer rates, could be attributed to the decrease in soil N and the high C:N ratio of the cover crop residue (Tables 2.3 and 2.6). It is generally accepted that crop residues with high C:N ratio (more than 25:1) decompose more slowly than those with a low C:N ratio (Fageria et al., 2005), and consequently, soil N can still be immobilized and not readily available for the succeeding crop in the rotation (Dabney et al., 2001). This scenario can result in reduced crop yields if sufficient N is not supplied to the following crop, especially a cereal crop (Blanco-Canqui et al., 2015). In addition, it is important to highlight that the 0-N control treatment only received fertilizer N during the wheat phase of the rotation, and thus, the inherent soil mineral N level might

have decreased over time. With low levels of soil N, it is expected to find a significant response to N fertilizer rates.

The effects of CCs on sorghum grain yield were more evident at the 0-N and 45-N. Where no fertilizer was applied, LMS increased sorghum yield by 10.6% relative to CF and CL, 15.8% relative to DR, and 69.2% relative to SS. At 45-N, grain yield of sorghum planted after LMS was still significantly greater than after CL (11.4%), DR (12.7%), and SS (45%). No significant difference was observed between LMS and DSB at both 0-N and 45-N. Thus, the increased sorghum yields following a summer legume cover crop and DSB could be due to the increase in soil N availability to sorghum compared with CF and non-legume CCs (Table 2.6). Blanco-Canqui et al. (2012) found that near-surface soil total N increased by 258 kg ha<sup>-1</sup> under LMS compared with a no cover crop treatment. Several other studies have found significant yield responses of cereal crops following legume CCs when compared to CF or non-legume CCs (Balkcom and Reeves, 2005; Blevins et al., 1990; Reinbott et al., 2004; Schomberg et al., 2007). The results of this study are in agreement with other research, suggesting that legume CCs are useful tools to enhance N availability and grain yields in a cereal-cereal cropping sequence.

Although total N uptake was greater in 2015 than in 2014, the cover crop treatments had similar effects in both years. The greater N accumulation of sorghum in 2015 could be attributed to the greater ANM that occurred during the growing season (Table 2.6). The higher precipitation coupled with higher SOM in 2015 may have favored SOM decomposition and consequent N mineralization, increasing N availability to the sorghum plants. However, SS and DR reduced total N uptake by approximately 51 and 14%, on average, when compared to LMS and DSB, respectively. Similar results were reported by Salmerón et al. (2010) under irrigated corn comparing legume and non-legume CCs. The authors concluded that the N uptake reduction after

barley (*Hordeum vulgare* L.) and DR, when compared to hairy vetch, was due to an N deficiency caused by insufficient N mineralization from the cover crop due to high C:N ratio (barley) and/or lack of synchronization with corn N uptake (DR).

### ***Components of nitrogen use efficiency***

The NUE and components of N use can be defined as the maximum economic yield produced per unit of N applied, absorbed, or utilized by the plant to produce grain and dry matter (Baligar and Fageria, 2015). In general, NUE, NUpE, and NUtE were higher at lower N rates and decreased at higher N rates. These results indicate that the increased soil N supply and/or the increased N uptake by sorghum plants were not associated with a significant increase in grain yield (Fig 2.9). Muchow (1998) reported that with high N supply, N uptake is higher than the minimum required for a given yield level, and this excess N (called luxury N) uptake usually results in higher leaf and stem N concentration at maturity. This concept also explains the higher NUtE of sorghum planted after SS at 90-N and 180-N. In a recent study of the functional dynamics of the N balance of sorghum, Van Oosterom et al. (2010) found that during the early stages of grain filling, grain N demand was met predominantly through N translocation from the stem and rachis, and only under N stress did leaf N translocation occurred. Because SS reduced N availability to sorghum even at higher N rates, more N was translocated to produce grain, resulting in increased NUtE.

Although NUE and NUtE were greater in 2015 than in 2014, the cover crop treatments had similar effects in both years. When averaged across years, our results showed evidence that all CCs and DSB were improving N efficiency when compared to CF. The findings of this study are in agreement with review studies regarding best management practices to improve NUE in crop production reported in the literature (Kramberger et al., 2009; Ladha et al., 2005; Mishra and Patil, 2015; Robertson and Vitousek, 2009). In these studies, the authors emphasize that CCs increase



NUE by reducing N losses, providing N to the subsequent cash crop, and improving nutrient cycling.

The NHI index measures the partitioning of total plant N into grain, which provides an indication of how efficiently the plant utilized acquired N for grain production (Fageria, 2014). The lower NHI values at higher N rates could be the result of the luxury N uptake that is accumulated in the leaves and stems at maturity. Considering that optimum economic grain yield occurred at 90-N for most cover crop treatments, the CF treatment slightly decreased NHI of sorghum. Because NHI was positively associated with efficient utilization of N ( $R^2 = 0.4048$ ;  $P < 0.0001$ ), these results indicated better translocation of N from tissues to grain, and thereby improving NUE of sorghum planted after CCs and DSB when N fertilizer rate matches the minimum required for optimum grain yield.

The PFP is a simple production efficiency expression that can be calculated by any farm that keeps records of inputs and yields, and its typical level for cereal crops (primarily maize, rice, and wheat) ranges from 40 to 90 kg grain  $\text{kg}^{-1}$  N applied, as described by Dobermann (2007). At the optimum N rate (90-N), our results showed that PFP values for grain sorghum are in agreement with the data reported in the literature for other cereal crops, ranging from 66.8 to 82.8 kg grain  $\text{kg}^{-1}$  N applied. In addition, lower PFP levels suggest over application of N fertilizer (which occurred at 135-N and 180-N), and higher levels suggest that N supply is likely limiting productivity (as shown at 45-N) (Fixen et al., 2015).

### ***Nitrogen fertilizer replacement value***

The negative NRFV provided by most of the cover crop treatments in 2015 was mainly due to the greater sorghum grain yield following CF in that year, especially at lower N rates (data not shown). This data indicates that, with the exception of LMS and DSB, all the other CCs

somehow reduced N availability when compared to the CF. The N contribution of CCs to the following crop is strongly influenced by environmental and management factors (Reeves, 1994). In addition, the efficiency of the soil to supply N to plants is strongly influenced by immobilization and mineralization with changing climate and environment (Raun et al., 1998). It is possible that the residual N (N fertilization from previous crop, residue decomposition, and SOM mineralization) supplied substantial amounts of the N demanded by the sorghum plants, particularly in year where climate conditions (temperature and rainfall) was ideal for enhancing crop yields (Fig 2.1).

Timing of N release could also impact NFRV of CCs. Vyn et al. (2000) emphasize that the full benefit of using CCs will be dependent on the synchrony between cover crop N mineralization and N demand of the subsequent crop. Daikon radish grows fast during the fall and it immobilizes less N than some cereal CCs, and consequently, much of the N taken up can become available for uptake by the following crop in early spring (Clark, 2007, pp. 82). Because grain sorghum was planted in late May through June, the negative values of NFRV provided by daikon radish suggest that potentially the N was mineralized before the peak of N demand by sorghum plants. Our results also indicate that, if the goal is to improve N supply, either LMS or DSB are suitable in this cropping system, providing more than 28 kg N ha<sup>-1</sup> to the following grain sorghum crop, on average.

## **Conclusions**

The cover crop performance appeared to be highly dependent on the amount of precipitation received after planting. If water is not limited, both LMS and CL can produce adequate biomass and significant amounts of N during the traditional summer fallow period.

After 9 years of no-till management and 3 full cycles of the rotation, sorghum growth and development was affected by previous type of cover crop and N management. The effects of CL

and DR on the subsequent sorghum yield and N uptake was similar and negative to the CF treatment, respectively. The use of a high biomass non-legume cover crop (such as SS) after winter wheat likely resulted in N immobilization during the sorghum phase and consequently reduced N uptake and grain yield. On average, DSB and LMS were able to provide up to one-third and one-half of the N required for maximum economic grain yield, respectively.

All components of NUE tended to decrease with increasing N fertilizer rates. However, the CF treatment slightly decreased N<sub>utE</sub> and NHI at 90-N. These results show evidence that all CCs and DSB are improving N efficiency when N fertilizer rate matches the minimum required for maximum crop yield.

The findings of this study support our initial hypotheses that non-legume CCs would reduce N availability during the sorghum phase, resulting in decreased N uptake and sorghum grain yield. In addition, these results suggest that summer legume CCs or DSB have the potential to replace the traditional CF, improving management of N resources in a wheat-sorghum cropping sequence. The strategic use of summer legume CCs can potentially decrease the amount of N fertilizer required to maximize sorghum yields.

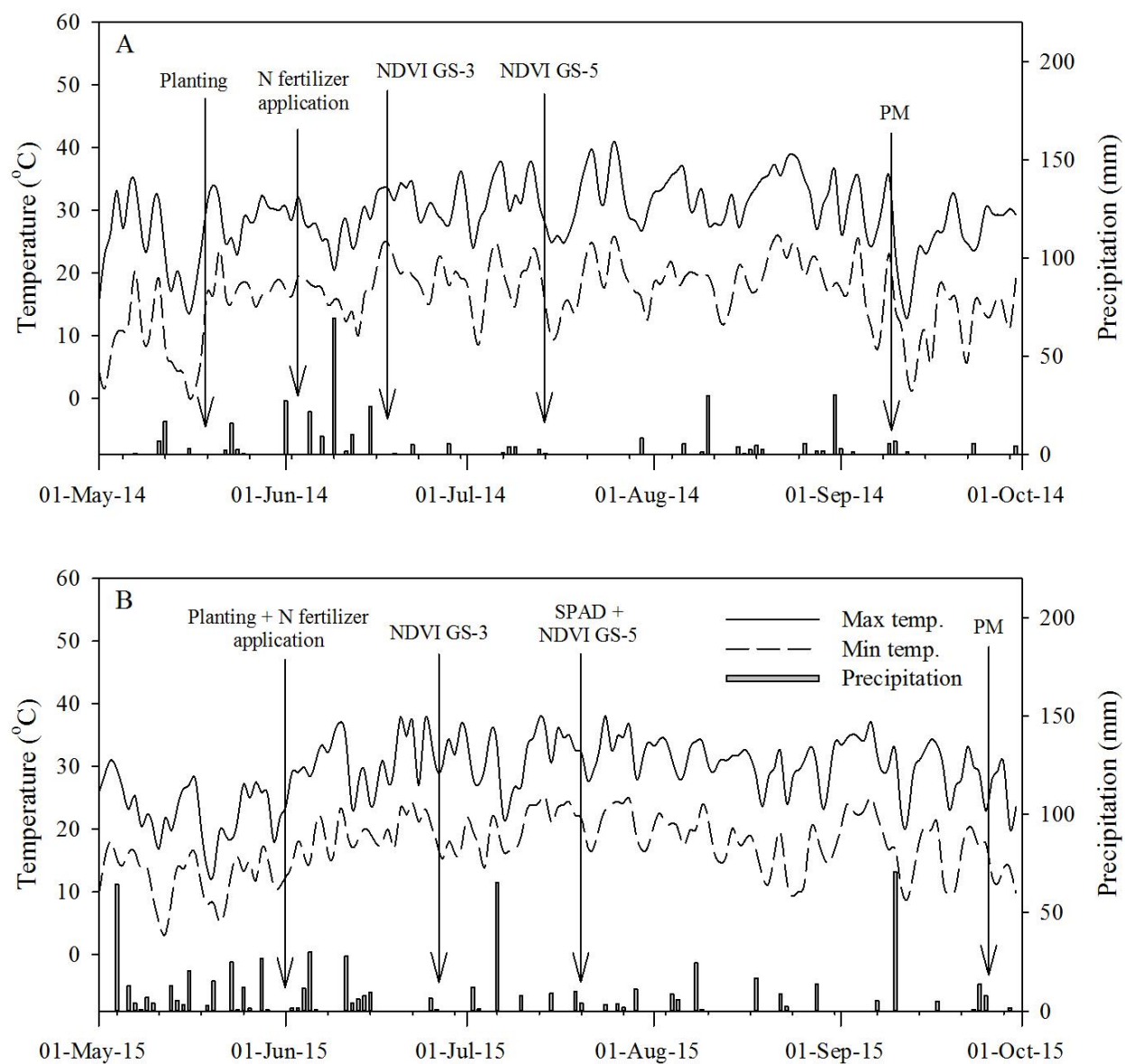
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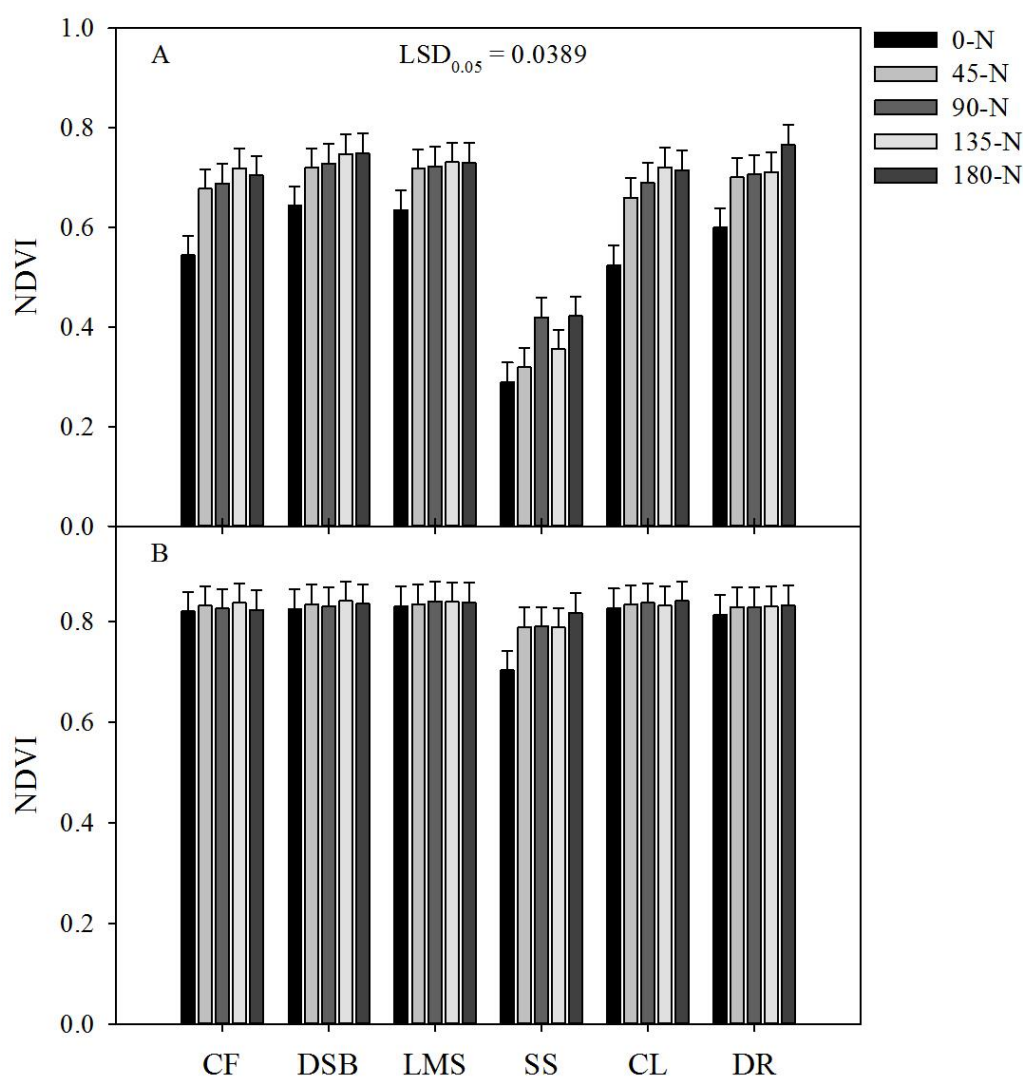
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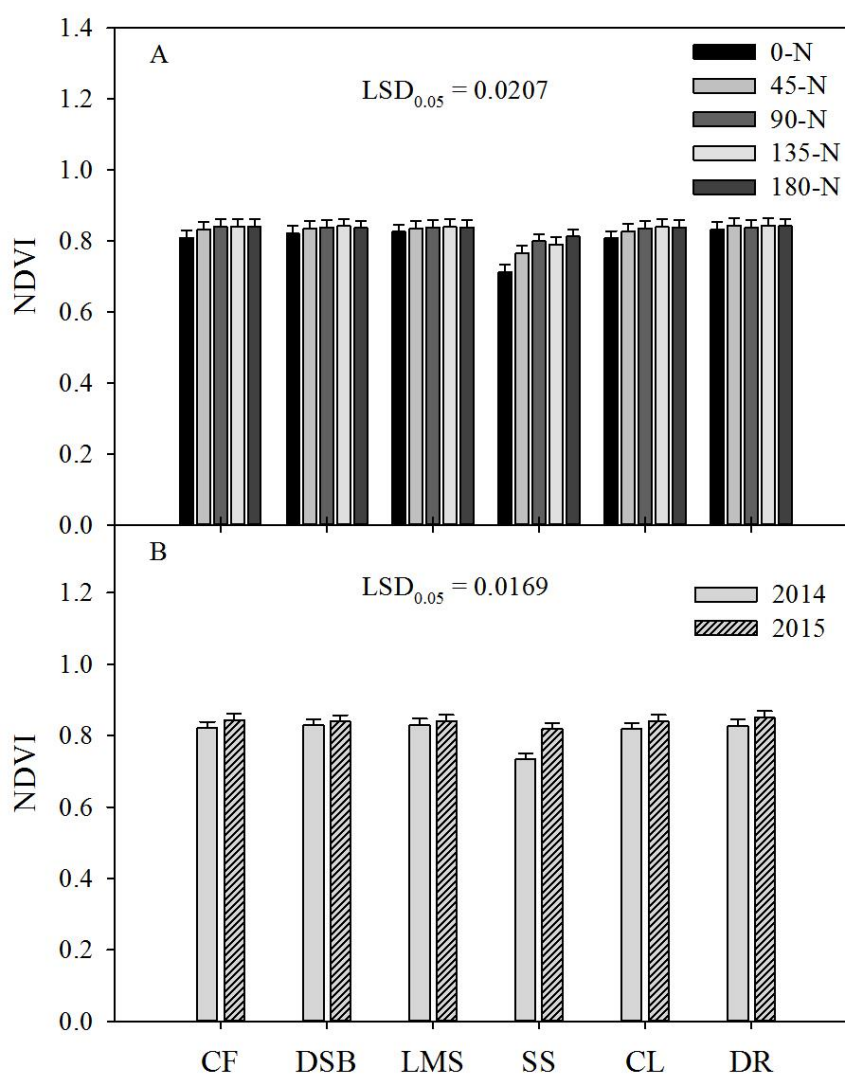


**Figure 2.1.** Daily maximum and minimum temperature, and precipitation during the sorghum grain growing season in 2014 (A) and 2015 (B). (GS, growth stage; N, nitrogen; NDVI, Normalized Difference Vegetation Index; SPAD, chlorophyll index; PM, physiological maturity).

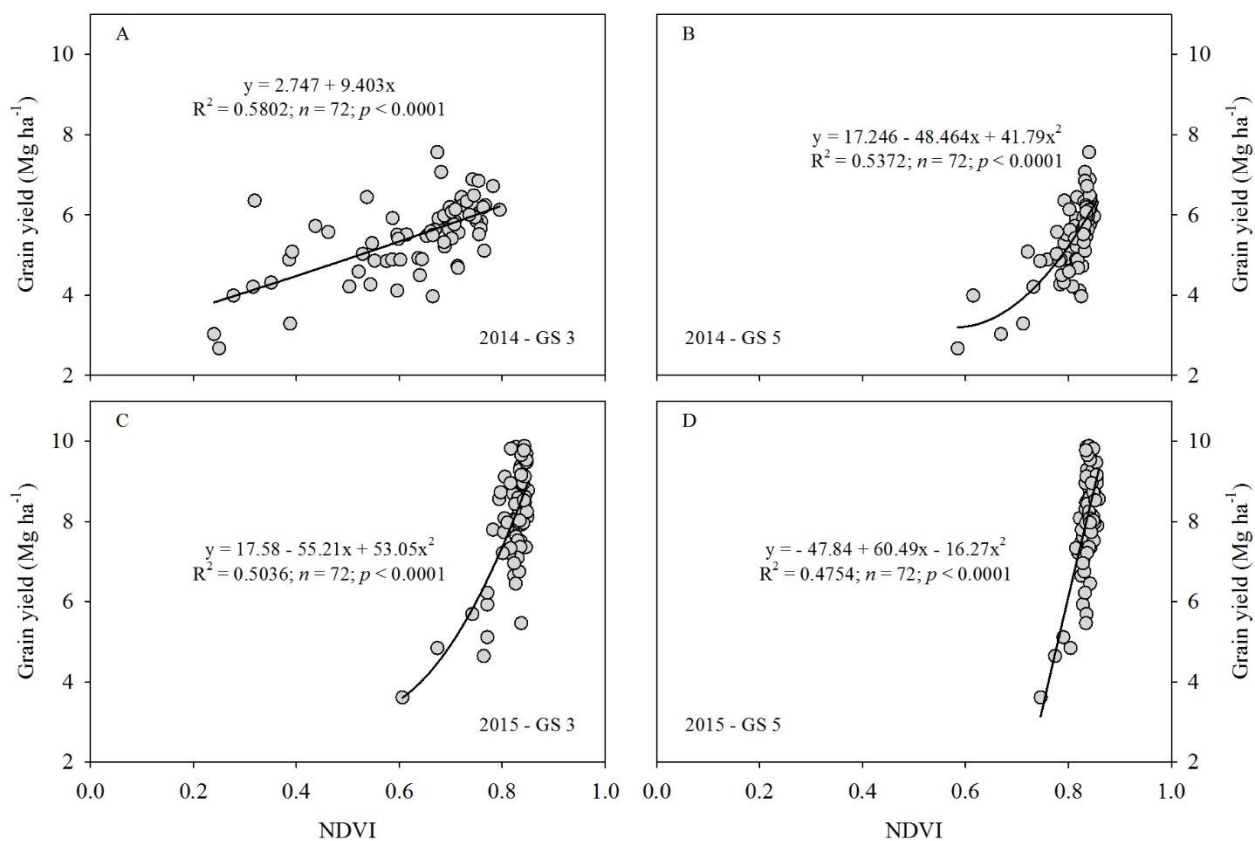




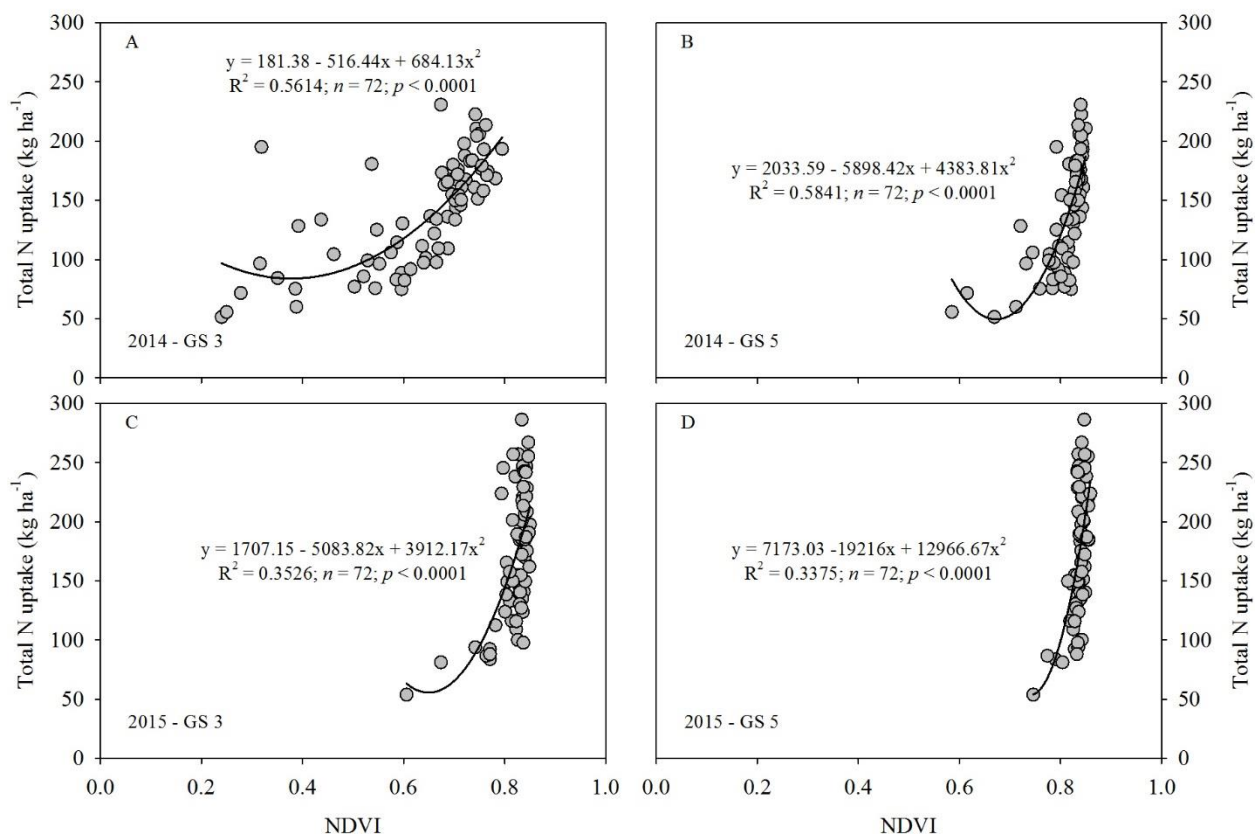
**Figure 2.2.** Normalized Difference Vegetation Index (NDVI) values at growth stage 3 as affected by cover crop and nitrogen (N) fertilizer rate on sorghum grown in 2014 (A) and 2015 (B). Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



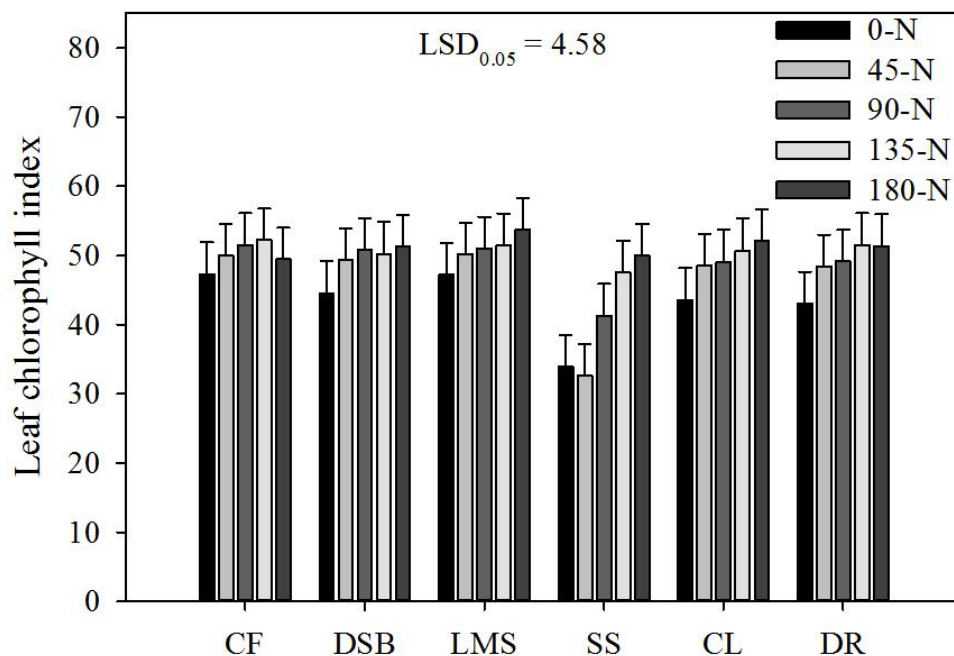
**Figure 2.3.** Interaction effects of cover crop by nitrogen (N) rate (A) and cover crop by year (B) on Normalized Difference Vegetation Index (NDVI) values at growth stage 5 of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



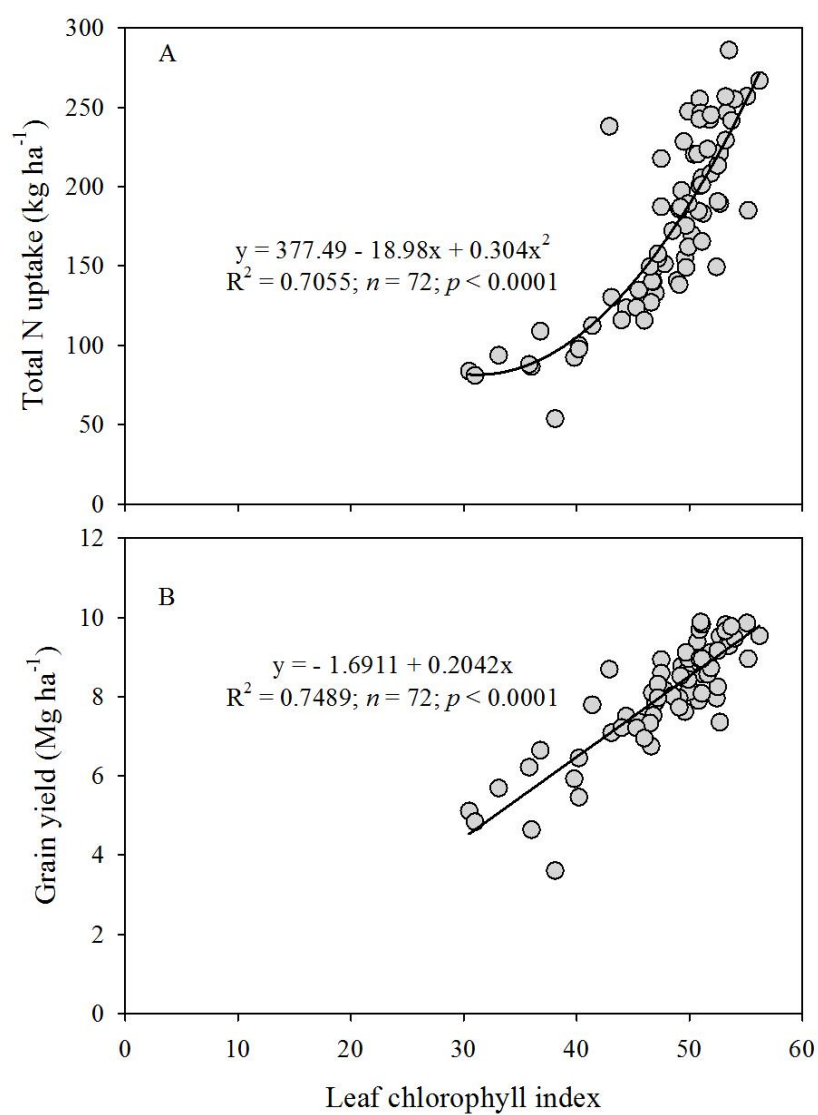
**Figure 2.4.** Relationship between sorghum grain yield and Normalized Difference Vegetative Index (NDVI) at growth stage 3 in 2014 (A), growth stage 5 in 2014 (B), growth stage 3 in 2015 (C), growth stage 5 in 2015 (D). (GS, growth stage).



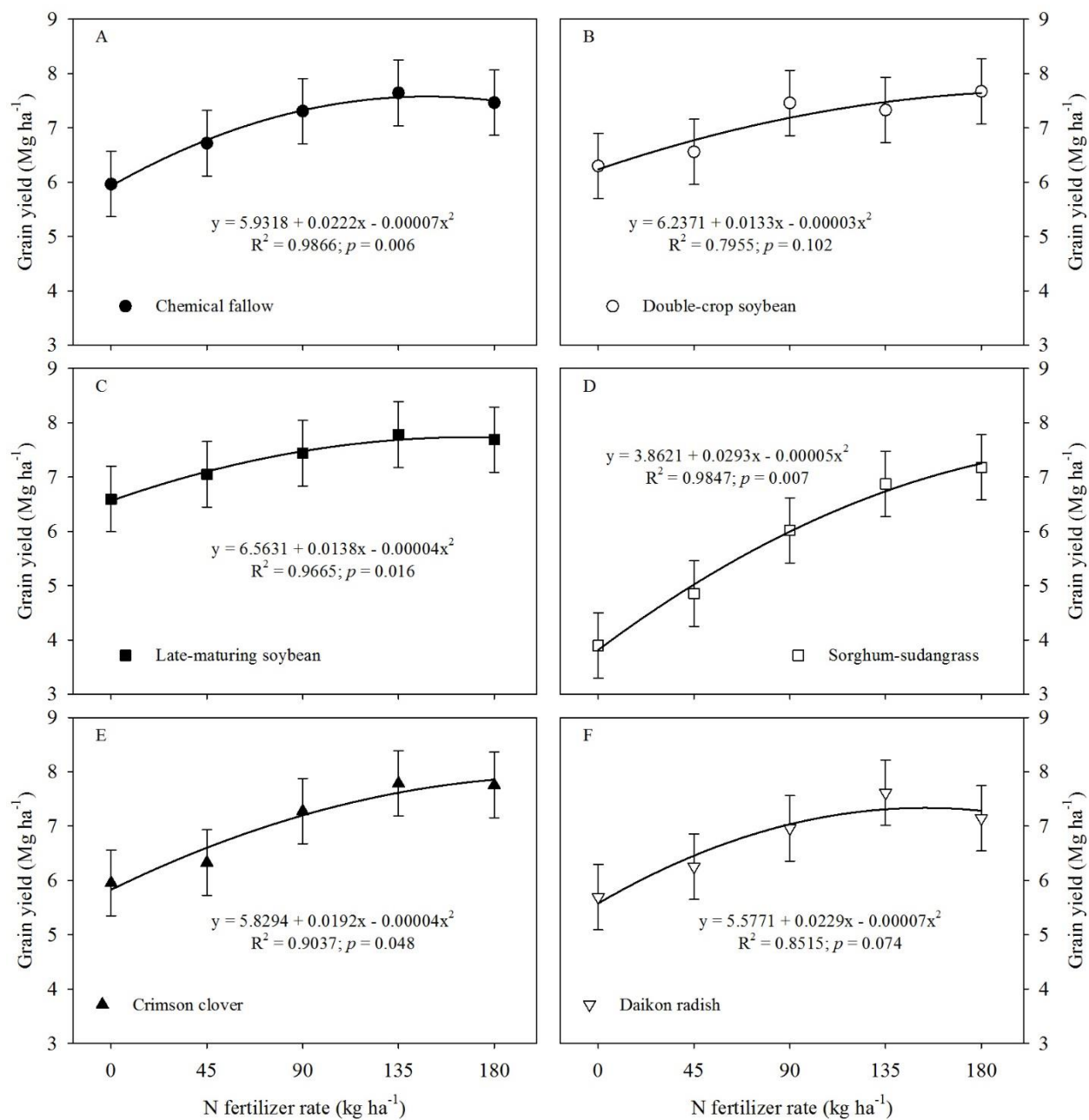
**Figure 2.5.** Relationship between total nitrogen (N) uptake at physiological maturity and Normalized Difference Index Vegetation (NDVI) at growth stage 3 in 2014 (A), growth stage 5 in 2014 (B), growth stage 3 in 2015 (C), growth stage 5 in 2015 (D). (GS, growth stage).



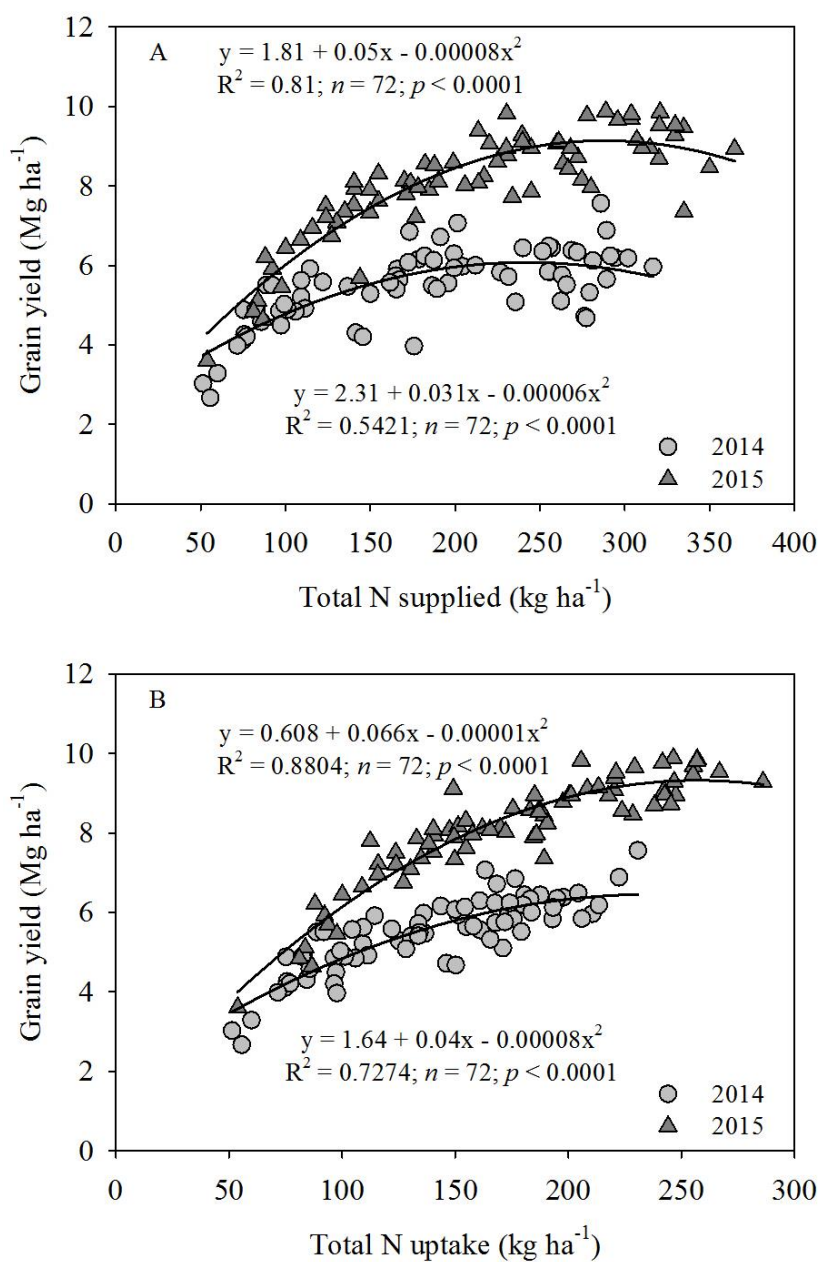
**Figure 2.6.** Leaf chlorophyll index as affected by cover crop and nitrogen (N) rates in sorghum grown in 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



**Figure 2.7.** Relationship between leaf chlorophyll index and total nitrogen (N) uptake at physiological maturity (A) and sorghum grain yield (B) in 2015.

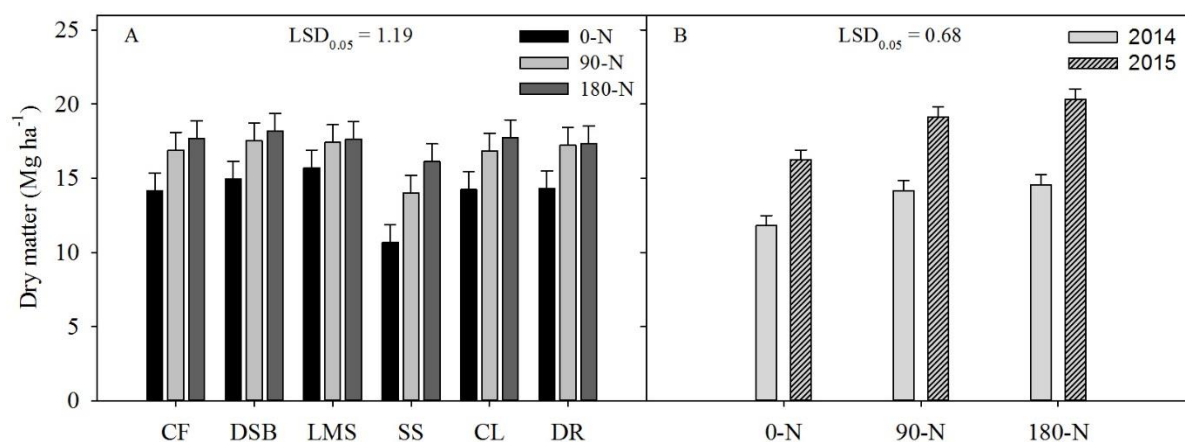


**Figure 2.8.** Effects of cover crop and nitrogen (N) rate on sorghum grain yield across both years. Vertical bars indicate the 95% confidence interval.

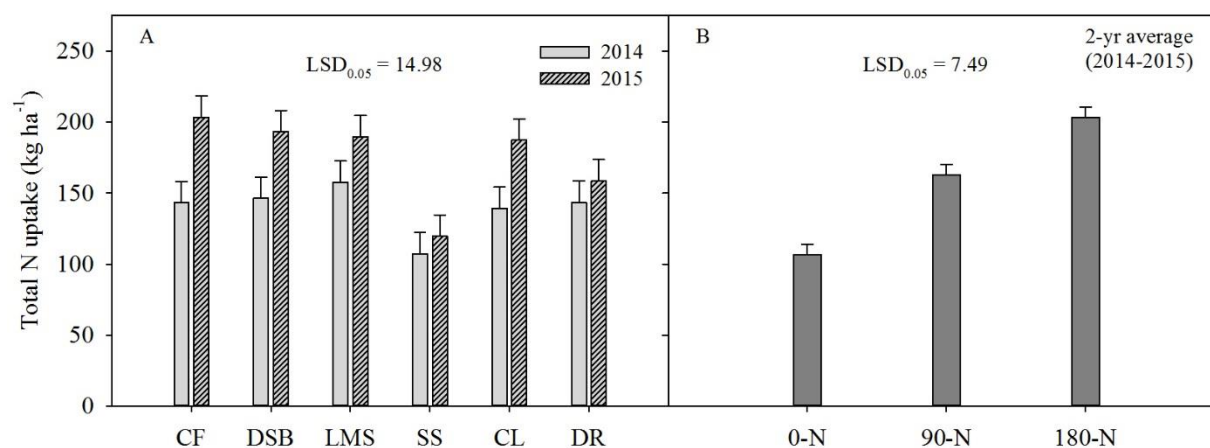


**Figure 2.9.** Relationship between grain yield and total nitrogen (N) supplied (A) and total N uptake (B) on sorghum grown in 2014 and 2015. (Total N supplied was estimated as the N fertilizer applied plus N supplied by the soil).

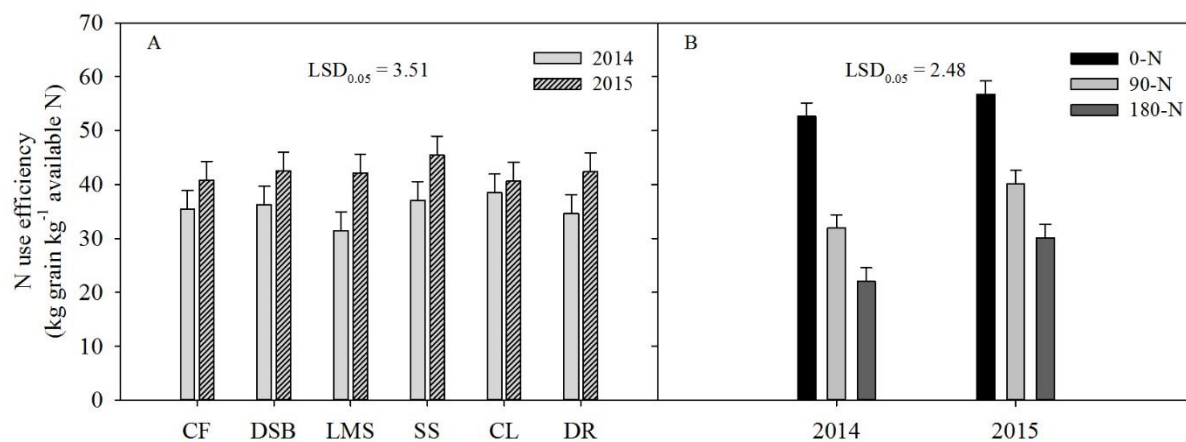




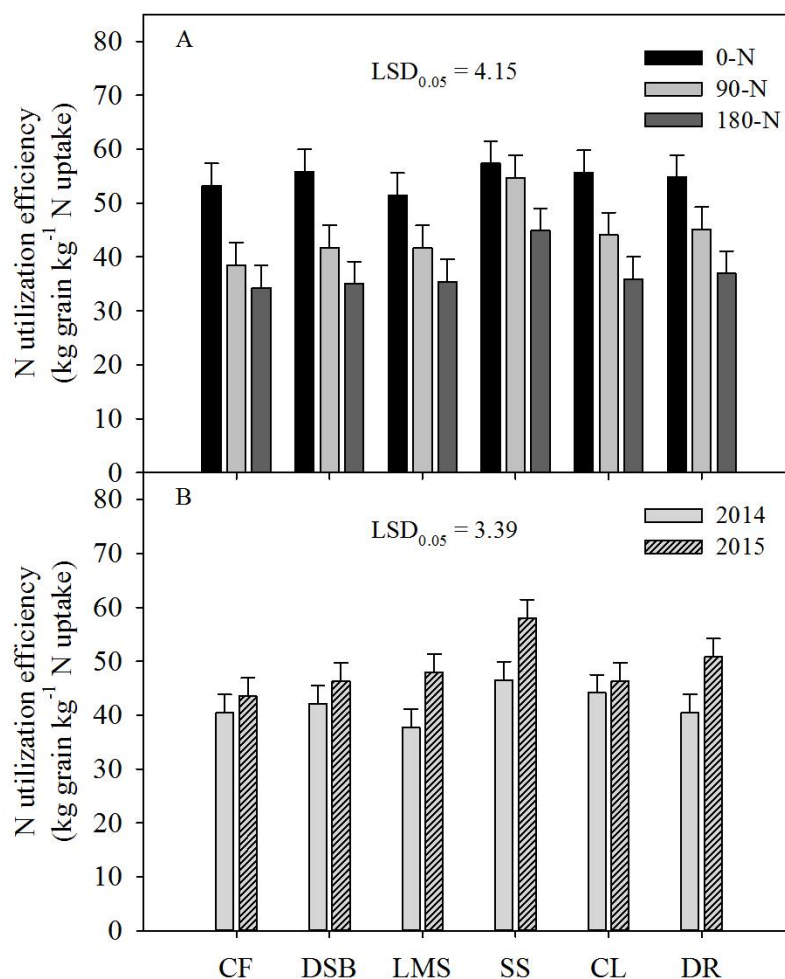
**Figure 2.10.** Interaction effects of cover crop by nitrogen (N) rate (A) and N rate by year (B) on the average dry matter production of sorghum in 2014 and 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



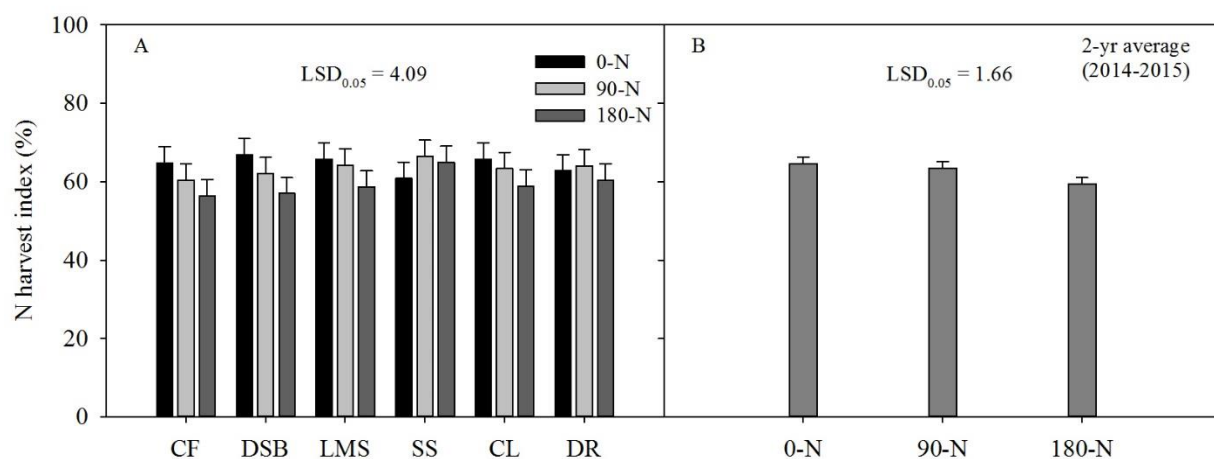
**Figure 2.11.** Effects of cover crop (A) and nitrogen (N) rates (B) on total N uptake at physiological maturity of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



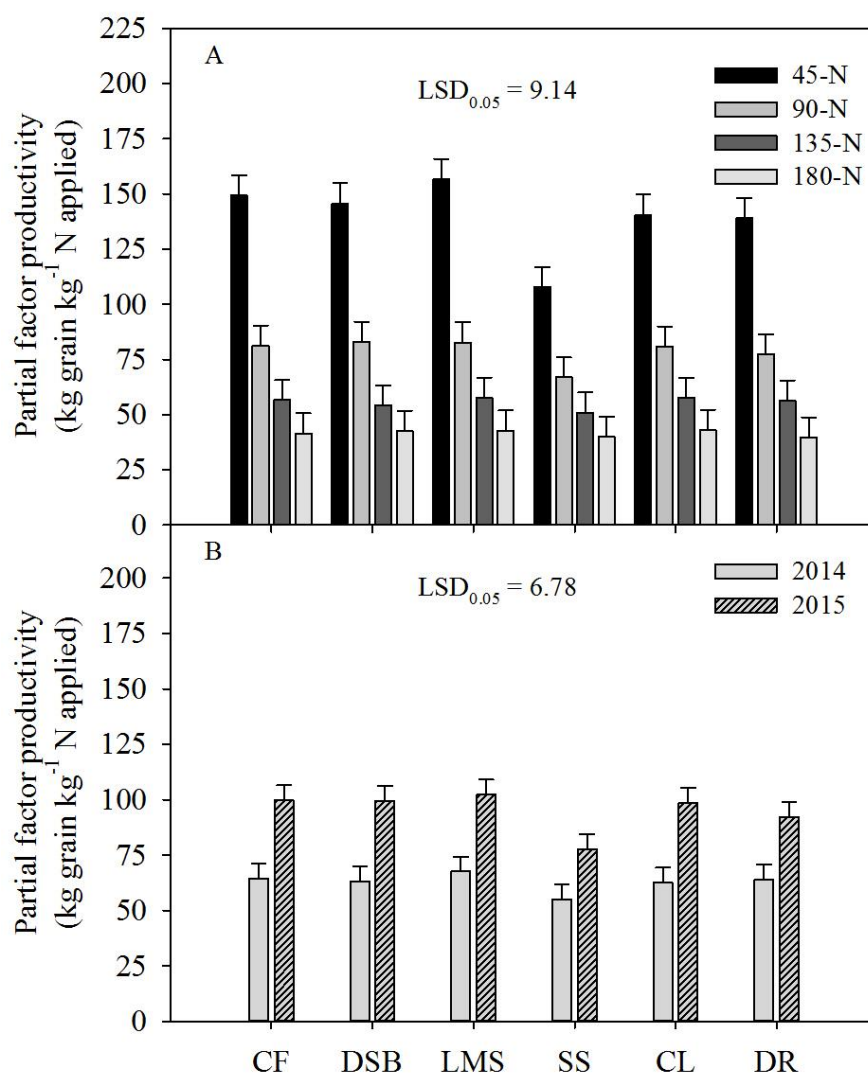
**Figure 2.12.** Effects of cover crop by year (A) and nitrogen (N) rate by year (B) on N use efficiency of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



**Figure 2.13.** Interaction effects of cover crop by nitrogen (N) rate (A) and cover crop by year (B) on N utilization efficiency of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



**Figure 2.14.** Nitrogen (N) harvest index of sorghum as affected by cover crops and N rates across both years. Vertical bars indicate the Fisher’s LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).



**Figure 2.15.** Interaction effects of cover crop by nitrogen (N) rate (A) and cover crop by year (B) on partial factor productivity of sorghum grown in 2014 and 2015. Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; LMS, late-maturing soybean; SS, sorghum-sudangrass; CL, crimson clover; DR, daikon radish.).

**Table 2.1.** Average monthly temperature and precipitation at Ashland Bottoms Research Farm in 2013, 2014, and 2015.

Month	Precipitation				Temperature			
	2013	2014	2015	Normal§	2013	2014	2015	Normal§
	----- mm -----				----- °C -----			
Jan	9.1	0.5	22.1	16.0	1.2	-2.9	-0.6	-1.7
Feb	19.5	22.9	10.2	27.0	1.2	-3.3	-2.5	1.1
Mar	25.7	31.1	4.2	63.0	4.3	4.2	8.1	6.5
Apr	87.6	89.1	67.6	81.0	10.0	9.5	13.9	12.5
May	99.0	49.5	218.7	129.0	17.8	19.3	17.6	18.4
June	88.4	176.8	107.2	145.0	23.9	23.8	24.9	23.7
July	36.8	21.1	128.0	112.0	25.2	24.8	26.4	26.7
Aug	23.9	91.4	81.0	105.0	25.1	26.3	23.8	25.6
Sept	104.7	29.0	104.9	87.0	22.7	20.3	23.3	20.5
Oct	110.0	74.4	15.5	68.0	13.4	14.7	15.0	13.6
Nov	10.4	1.3	110.5	44.0	5.6	3.2	9.5	6.2
Dec	1.8	54.1	82.8	27.0	-2.1	1.7	3.7	-0.5
Total	616.9	641.2	952.6	904.0	-	-	-	-

§30-yr average, 1981 – 2010 Normal.

**Table 2.2.** Selected soil chemical properties at the 15-cm depth taken during the spring before sorghum planting in 2014 and 2015.

Year	pH	Mehlich-3 P	Exchangeable K	Organic matter
		----- mg kg <sup>-1</sup> -----		g kg <sup>-1</sup>
2014	6.1	34	340	25.8
2015	6.2	19	321	33.4

P, phosphorus.

K, potassium.



**Table 2.3.** Total aboveground dry matter production, nitrogen (N) content, N concentration, and carbon to N ratio (C:N) of summer and winter cover crops at termination date.

Cover crop	Dry matter (Mg ha <sup>-1</sup> )	N content (kg ha <sup>-1</sup> )	N concentration (g kg <sup>-1</sup> )	C:N ratio
----- 2013-14§ -----				
Late-maturing soybean	1.6 b†	47.9 b	29.3 a	14:1 b
Sorghum-sudangrass	6.5 a	67.7 a	10.6 b	39:1 a
Crimson clover	-	-	-	-
Daikon radish	-	-	-	-
----- 2014-15 -----				
Late-maturing soybean	3.7 b	99.3 a	27.1 a	16:1 b
Sorghum-sudangrass	6.7 a	75.5 b	11.3 d	39:1 a
Crimson clover	3.3 bc	79.5 b	23.6 b	18:1 b
Daikon radish	2.7 c	42.3 c	15.7 c	24:1 c

§Crimson clover and daikon radish were not sampled in 2013-14, therefore they were reported as missing values.

†Treatment means within column for a given year followed by different letter are significantly different ( $P \leq 0.05$ ).

**Table 2.4.** ANOVA significance levels for the effect of cover crops, nitrogen rate, year, and their interactions on Normalized Difference Vegetation Index (NDVI) at Stage 3 and 5, and leaf chlorophyll index of grain sorghum grown in 2014 and 2015.

Source of variation	NDVI GS-3	NDVI GS-5	Chlorophyll index
Cover crop (CC)	< 0.0001	< 0.0001	< 0.0001
N rate (N)	< 0.0001	< 0.0001	< 0.0001
Year (Y)	< 0.0001	< 0.0001	-§
CC x N	0.017	< 0.0001	0.001
CC x Y	< 0.0001	< 0.0001	-
N x Y	< 0.0001	< 0.0001	-
CC x N x Y	0.001	0.273	-

GS-3, growth Stage 3.

GS-5, growth Stage 5.

§Leaf chlorophyll index was measured only in 2015, therefore there is no effect of year and its interactions.

**Table 2.5.** ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, year, and their interactions on grain yield, dry matter production, and total N uptake of sorghum grown in 2014 and 2015.

<b>Source of variation</b>	<b>Grain yield</b>	<b>Dry matter</b>	<b>Total N uptake</b>
Cover crop (CC)	< 0.0001	< 0.0001	< 0.0001
N rate (N)	< 0.0001	< 0.0001	< 0.0001
Year (Y)	< 0.0001	< 0.0001	< 0.0001
CC x N	< 0.0001	0.047	0.334
CC x Y	0.0005	0.642	< 0.0001
N x Y	0.001	0.026	0.283
CC x N x Y	0.472	0.832	0.596

**Table 2.6.** Apparent nitrogen mineralization§ from each cover crop treatment during the sorghum growing season in 2014 and 2015.

Cover crop treatment	Year		Mean
	2014	2015	
	----- kg ha <sup>-1</sup> -----		
Chemical fallow	88.74 b†	124.01 ab	106.40 b
Double-crop soybean	98.20 ab	129.63 a	113.94 ab
Late-maturing soybean	117.59 a	136.95 a	127.29 a
Sorghum-sudangrass	59.63 c	73.84 c	66.76 c
Crimson clover	86.55 b	118.77 ab	102.69 b
Daikon radish	93.33 b	105.06 b	99.23 b

§ Apparent nitrogen mineralization was estimated from the total N uptake (stover + grain) from the zero N control plots.

† Treatment means within column followed by different letter are significantly different ( $P \leq 0.05$ ).

**Table 2.7.** ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, year, and their interactions on partial factor productivity (PFP), N use efficiency (NUE), N utilization efficiency (NUE), N uptake efficiency (NUpE), N recovery efficiency (N<sub>RE</sub>) and N harvest index (NHI) of grain sorghum grown in 2014 and 2015.

<b>Source of variation</b>	<b>PFP</b>	<b>NUE</b>	<b>NUE</b>	<b>NUpE</b>	<b>N<sub>RE</sub></b>	<b>NHI</b>
Cover crop (CC)	< 0.0001	0.019	< 0.0001	0.003	0.161	0.215
N rate (N)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.106	< 0.0001
Year (Y)	< 0.0001	< 0.0001	< 0.0001	0.025	0.478	< 0.0001
CC x N	< 0.0001	0.995	0.016	0.147	0.636	0.0005
CC x Y	0.011	0.029	< 0.0001	0.444	0.582	0.282
N x Y	< 0.0001	0.039	0.021	0.207	0.675	0.115
CC x N x Y	0.159	0.319	0.310	0.944	0.974	0.099

**Table 2.8.** Main effects of the cover crop, nitrogen (N) rates, and year on N uptake efficiency on sorghum grown in 2014 and 2015 in Ashland Bottoms, KS.

<b>Treatment</b>	<b>N uptake efficiency (%)</b>
<i>Cover crops</i>	
Chemical fallow	89.22 a§
Double-crop soybean	87.25 ab
Late-maturing soybean	83.77 ab
Sorghum-sudangrass	78.21 c
Crimson clover	85.79 ab
Daikon radish	82.78 bc
<i>N rates</i>	
0 kg N ha <sup>-1</sup>	100 a
90 kg N ha <sup>-1</sup>	82.80 b
180 kg N ha <sup>-1</sup>	70.72 c
<i>Year</i>	
2014	82.68 b
2015	86.33 a

§Treatment means within column followed by different letter are significantly different ( $P \leq 0.05$ ).

**Table 2.9.** Regression equations of sorghum grain yield as a function of nitrogen fertilizer rate for the chemical fallow treatment in 2014 and 2015.

Year	Regression	Equation	R <sup>2</sup>	CV for (y)	Pr > F
2014	Linear	$y = 4.70961 + 0.0094x$	0.51	11.0	0.0004
	Quadratic	$y = 4.519 + 0.0178x - 0.00004x^2$	0.58§	10.9	0.001
2015	Linear	$y = 7.5459 + 0.00977x$	0.52	7.61	0.0024
	Quadratic	$y = 7.122 + 0.0285x - 0.000104x^2$	0.63§	6.38	0.0009

§ Adjusted R<sup>2</sup>.

CV, coefficient of variation.

**Table 2.10.** Estimated nitrogen fertilizer replacement value provided to grain sorghum by cover crops and double-crop soybean in 2014 and 2015.

Cover crop treatment	Year		Mean
	2014	2015	
	----- kg ha <sup>-1</sup> -----		
Double-crop soybean	52.4	4.8	28.6
Late-maturing soybean	61.6	26.7	44.1
Sorghum-sudangrass	-60.7	-74.6	-67.6
Crimson clover	35.3	-19.8	7.75
Daikon radish	6.9	-20.5	-6.8



## Chapter 3 - Replacing chemical fallow with cover crops in no-till cropping systems: Effects on nitrous oxide emissions

### Abstract

In the U.S., 9% of the total greenhouse gas emissions in 2013 were from the agriculture sector. Of this, soil management accounted for 74% of total nitrous oxide (N<sub>2</sub>O) sources, largely related to nitrogen (N) fertilizer input. Growing cover crops (CCs) in rotation with cash crops has been proposed to reduce soil N<sub>2</sub>O emissions by scavenging excess mineral N after cash crop harvest and by reducing N fertilizer requirements for the following cash crop. We evaluated the effect of CCs and N fertilization on soil nitrate (NO<sub>3</sub><sup>-</sup>) and N<sub>2</sub>O emissions from a wheat (*Triticum aestivum* L.) – sorghum [*Sorghum bicolor* (L.) Moench] cropping sequence in northeastern Kansas. Two CCs [sorghum-sudangrass (*Sorghum vulgare* var. sudanese), daikon radish (DR) (*Raphanus sativus* L.)] and a double-crop soybean (DSB) [*Glycine max* (L.) Merr] were planted following wheat harvest. Fertilizer N was banded at three rates (0, 90 and 180 kg ha<sup>-1</sup>) following sorghum planting. Soil N<sub>2</sub>O fluxes were measured by the closed-static chamber method during the 2014-15 and 2015-16 growing seasons. The effect of fallow and N management on emissions during the wheat phase was not consistent in both growing seasons, suggesting that the residual effect of the treatments may occur only in wetter years or that the lasting cover crop benefits were minimal in the wheat planted two years after CCs were grown. Soil NO<sub>3</sub><sup>-</sup> was consistently greater in chemical fallow (CF), with little temporal change in the remaining cover crop treatments during the fallow period. Although the CCs and DSB decreased N<sub>2</sub>O emissions by 65% during the fallow period compared to CF, they increased emissions during the sorghum phase when N was applied (except DR). Sorghum-sudangrass was the only cover crop that significantly decreased the N<sub>2</sub>O efficiency of the cropping system, increasing yield-scaled N<sub>2</sub>O emission by 1.7-fold, on average.

Also, N<sub>2</sub>O dynamics appear to be more sensitive when synthetic N fertilizer is applied in the system, perhaps indicating that N fertilization might be the overriding factor. Potential impacts of greater soil N<sub>2</sub>O emissions from CCs should be evaluated in context with other beneficial agroecosystem services of cover cropping such as reduced soil erosion, increased soil and water quality, increased weed suppression, etc.

Abbreviations: 0-N, 0 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CCs, cover crops; FIEF, fertilizer induced emission factor; N<sub>2</sub>O, nitrous oxide; YSNE, yield-scaled N<sub>2</sub>O emissions.

## **Introduction**

The global atmospheric nitrous oxide (N<sub>2</sub>O) concentration has increased from a pre-industrial value of 270 to 319 ppb in 2013 (IPCC, 2014). Although its atmospheric concentration is lower than that of carbon dioxide (CO<sub>2</sub>), N<sub>2</sub>O has a global warming potential 298 times higher than CO<sub>2</sub> (Solomon et al., 2007). In the United States, 9% of the total greenhouse gas emissions were from the agriculture sector, and of this, soil management accounted for 74% of the total anthropogenic N<sub>2</sub>O sources, mainly because of synthetic nitrogen (N) fertilizer used in crop production (USEPA, 2015; Van Groenigen et al., 2010).

Nitrous oxide gas is produced in soil by microbial metabolism primarily during the process of denitrification and, to a lesser extent, during nitrification (Klefoth et al., 2012). In denitrification, nitrate (NO<sub>3</sub><sup>-</sup>) is progressively reduced to N<sub>2</sub>O during organic C oxidation under anaerobic conditions (Aulakh et al., 1992). Denitrification-driven N<sub>2</sub>O emission under field conditions is complex and difficult to manage because it is controlled by the interaction of many factors such as soil pH, texture, temperature, availability of NO<sub>3</sub><sup>-</sup> and soluble C, soil oxygen, as well as water status (Firestone, 1982).

It is well documented that the addition of synthetic N fertilizer has increased N<sub>2</sub>O emissions to a greater than background levels because of its direct impact on soil mineral N availability (Snyder et al., 2009). However, response curves for N<sub>2</sub>O flux as a function of N fertilizer are highly variable and contrasting results can be found in the literature. Shcherbak et al. (2014) conducted a global meta-analysis on 78 published data sets and found that N<sub>2</sub>O response to N input was exponential. Kim et al. (2013) reported 18 published papers with non-linear response (exponential or hyperbolic) of N<sub>2</sub>O emissions as a function of N fertilizer, but the relationship was linear in four datasets with at least four different levels of N input. Nevertheless, this suggests that N<sub>2</sub>O emissions increased rapidly once soil mineral N exceeded crop N demand. The use of appropriate N rates can help mitigate N<sub>2</sub>O emissions by minimizing the accumulation of NO<sub>3</sub><sup>-</sup> surplus in the soil and/or when crop recovery of the applied N is low (Snyder et al., 2009; Van Groenigen et al., 2010). Hoben et al. (2011) found that for the two N fertilizer rates that exceeded the rate recommended for maximum economic return, the average N<sub>2</sub>O fluxes were 43 and 115% greater than the flux at the recommended rate.

Post-harvest soil NO<sub>3</sub><sup>-</sup> accumulation is another major concern regarding N<sub>2</sub>O emissions in agricultural lands. Replacing chemical fallow with cover crops (CCs) can reduce N<sub>2</sub>O emissions during the fallow period by taking up the excess mineral N after cash crop harvest that otherwise would be readily available for denitrification (Scharf, 2015). Legume CCs can indirectly reduce N<sub>2</sub>O emissions by reducing N fertilizer requirements for the following cash crop (Blanco-Canqui et al., 2015). However, CCs also can increase N<sub>2</sub>O after termination due to biomass decomposition, but the magnitude of increase depends on the quantity and quality of the crop residues (Gomes et al., 2009). The addition of legume cover crop residues (with low C:N ratio) to the soil is expected to increase N<sub>2</sub>O emissions when compared to a non-legume cover crop residue (Baggs et al., 2003),

largely because of an increase in the mineralization rate, resulting in increased availability of  $\text{NO}_3^-$  and C substrate for denitrification. In addition, when CCs are killed shortly before N application, mineralizable C from their residues may stimulate denitrification and  $\text{N}_2\text{O}$  emissions (Petersen et al., 2011).

Even though, contrasting effects of CCs on  $\text{N}_2\text{O}$  emissions have been reported, perhaps reflecting differences in cover crop species, crop rotation, residue type, soil and climate conditions, etc. Jackeri et al. (2009) found no significant effect of a winter rye (*Secale cereal* L.) cover crop on cumulative  $\text{N}_2\text{O}$  emissions in a corn (*Zea mays*) – soybean [*Glycine max* (L.) Merr] rotation. Mitchell et al. (2013) found that winter rye decreased  $\text{N}_2\text{O}$  emissions when no N fertilizer was applied in a no-till corn-soybean rotation, but increased when N was applied. Petersen et al. (2011) found that daikon radish (*Raphanus sativus* L.) reduced  $\text{N}_2\text{O}$  emissions during the fallow period but emissions increased during the following cash crop. In a long-term no-till study in southern Brazil, Gomes et al. (2009) found greater cumulative  $\text{N}_2\text{O}$  emissions in corn following legume CCs [vetch (*Vigna sativa* L.), cowpea (*Vigna unguiculata* L. Walp), pigeon pea (*Cajanus cajan* L. Mill sp.), and lablab (*Dolichos lablab*)] than following a black oat (*Avena strigose* Schreb) non-legume cover crop.

Although CCs have been proposed as a strategy to both reduce N losses and mitigate  $\text{N}_2\text{O}$  emissions, there are no studies simultaneously assessing the effect of different types of CCs and varying N fertilizer rates in long-term crop rotations including grain sorghum [*Sorghum bicolor* (L.) Moench] in the Great Plains. To date, most studies have focused on the effects of N fertilizer rates on corn, wheat (*Triticum aestivum* L.), and soybean (Dusenbury et al., 2008; Hoben et al., 2011; Parkin and Kaspar, 2006; Venterea and Coulter, 2015). Identifying the optimum N fertilizer rate applied to grain sorghum, plus the selection of CCs adapted to climatic conditions could be

key strategies used to improve the sustainability of Great Plains cropping systems. Our general hypotheses were that (i) the use of CCs would reduce N<sub>2</sub>O emissions during the fallow period after wheat harvest and (ii) the legacy effects of CCs would reduce the cumulative N<sub>2</sub>O emissions regardless of the N rate during the sorghum cash crop phase of the rotation. The objectives of this study were (i) to define important periods of N<sub>2</sub>O losses throughout the cropping system, and (ii) to evaluate the effects of fallow management [chemical fallow (CF) vs. double-crop soybean (DSB), sorghum-sudangrass (SS) (*Sorghum vulgare* var. sudanese), and DR], N fertilization (0, 90, and 180 kg ha<sup>-1</sup>), and their interactions on N<sub>2</sub>O emissions in a three-year no-till winter wheat – sorghum – soybean rotation in northeastern Kansas. An additional objective was to evaluate the N<sub>2</sub>O efficiency of the cropping system by expressing emissions in relation to the agronomic parameters of grain sorghum.

## **Material and methods**

### ***Site description and experimental design***

This study was conducted within a long-term experiment established in 2007 at Ashland Bottoms Research Farm of Kansas State University (K-State), located approximately 8 km south of Manhattan, KS (39° 11' N lat, 96° 35' W long), at an altitude of approximately 311 m. The region has a humid subtropical climate (Köppen Climate Classification System: Cfa), with an average annual precipitation of 904 mm (30-yr average). January and June are the months with the lowest (16 mm) and highest (145 mm) precipitation, respectively. The annual mean temperature is 12.7°C. The soil was a moderately well-drained Wymore silty clay loam (fine, smectitic, mesic Aquertic Argiudoll), with 0 to 1% slopes.

The experiment consisted of a no-till, three-year cropping system of winter wheat – grain sorghum – soybean, where the CCs and DSB were grown after wheat harvest. Each component in

the rotation was present every year. Field experiments were conducted separately for the wheat, cover crop, and sorghum phases of the rotation. The study was conducted during the 2014-15 and 2015-16 growing seasons. The experimental design was a split-plot randomized complete block, with four replications. The CCs were the whole plots and N rates were the subplots. Each whole plot was 6 by 70 m and each subplot was 6 by 14 m. The cover crop treatments included SS and DR, plus a CF treatment as a control and a DSB treatment as a cash crop alternative. The N fertilizer treatments consisted of a control (zero N) and four N rates (45, 90, 135, and 180 kg ha<sup>-1</sup>) that were applied as 28% Urea Ammonium Nitrate (UAN) immediately after sorghum planting. The N<sub>2</sub>O measurements were made only on the plots receiving 0, 90, and 180 kg N ha<sup>-1</sup> (hereafter 0-N, 90-N, and 180-N).

### ***Cropping system management***

#### ***Winter wheat phase***

Winter wheat was planted immediately after soybean harvest on 20 Oct 2014 and 13 Oct 2015 with a target seeding rate of 115 kg ha<sup>-1</sup> on 19-cm rows using a John Deere 1590 no-till drill (Deere & Co., Moline, IL). Diammonium phosphate was applied at planting with wheat seed at a rate of 65 kg ha<sup>-1</sup>, supplying ~12 and 30 kg of N and phosphorous (P) ha<sup>-1</sup>. On 13 Mar 2015 and 26 Feb 2016, the wheat was top-dressed with 67 kg N ha<sup>-1</sup> soon after spring greenup using 28% UAN, applied in streams spaced every 10 cm. Weeds were controlled by applying glyphosate [N-(phosphonomethyl)glycine] at a rate of 1.67 kg a.i. ha<sup>-1</sup> the day after planting, and a herbicide in the form of 21 g a.i. ha<sup>-1</sup> of thifensulfuron-methyl plus 10.5 g a.i. ha<sup>-1</sup> of tribenuron-methyl (Harmony® Extra SG, DuPont) on 3 Mar 2015 and 10 Mar 2016, respectively. The wheat was harvested on 24 June 2015 and 22 June 2016.

#### ***Cover crop phase***

The CCs and DSB were planted immediately after wheat harvest on 3 July 2014 and 25 June 2015, with a target seeding rate of 23 kg ha<sup>-1</sup> for SS, 12 kg ha<sup>-1</sup> for DR, and 420,000 seeds ha<sup>-1</sup> for DSB in both years. Immediately after cover crop and DSB planting and before emergence, the plots were sprayed with glyphosate at a rate of 1.67 kg a.i. ha<sup>-1</sup>. The SS cover crop was terminated using a roller-crimper (I & J Manufacturing LLC, Gordonville, PA) at beginning of flowering (approximately 10 weeks after planting). Daikon radish was killed by freezing temperatures, which usually occurred during the month of December. The DSB plots were harvested with a plot combine on 7 Nov 2014 and 20 Oct 2015. On 20 Mar 2015 and 8 Apr 2016, all plots were sprayed with 1.46 kg a.i. ha<sup>-1</sup> of glyphosate plus 0.53 kg a.i. ha<sup>-1</sup> of 2,4-D (2,4-Dichloro-phenoxyacetic acid) to kill volunteer wheat and other winter annual weeds.

### ***Grain sorghum phase***

A medium-late maturity hybrid (DKS 53-67) was planted on 1 June 2015, with a target seeding rate of 119,000 seeds ha<sup>-1</sup>. Planting was performed using a White 6200 4-row planter (AGCO Corp., Duluth, GA) to a depth of 2.54 cm, with 76-cm row spacing. Nitrogen fertilizer rates were subsurface banded on 2 June 2015 using 28% UAN with a straight flat-coulter liquid fertilizer applicator to inject N fertilizer below the residue layer. No P or potassium (K) fertilizer were applied. Sorghum was sprayed with 1.25 kg a.i. ha<sup>-1</sup> of glyphosate and a herbicide in the form of 2.26 kg a.i. ha<sup>-1</sup> of acetochlor [2-chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl)acetamide] and 1.12 kg a.i. ha<sup>-1</sup> of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] (Degree Xtra®, Monsanto) within 2 days after planting to control post-emergence of weeds. When needed, hand weeding was also conducted throughout the growing season to maintain weed free plots.

After sorghum physiological maturity, dry matter (DM) yield was determined by hand harvesting plant samples from 0.76 m<sup>2</sup> area in the center of each plot on 28 Sept 2015. The plants were clipped just above the crowns and separated into two components: panicles and stover (stems + leaves), and weighed in the field. Sorghum stover was then chopped to 2.5 cm length using a chipper shredder (Model CS 3310, Cub Cadet, Valley City, OH), mixed thoroughly, and a representative subsample of about 300 g was collected for determination of DM yield and N concentration. Both panicles and stover were dried at 60°C in a forced-air oven until constant weight. The dried stover was ground to pass a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ). The panicles were threshed in a stationary thresher (Model SPVT, ALMACO, Nevada, IA) and the grain ground to a fine powder in the lab. Nitrogen concentration for both stover and grain samples were determined using the salicylic-sulfuric acid digestion method (Bremner and Mulvaney, 1982) at the K-State Soil Testing Laboratory. On 15 Oct 2015, sorghum was harvested from the two center rows of each plot with a modified 2-row Gleaner Model E-II combine (AGCO Corp, Duluth, GA) for yield determination. Grain moisture and test weight were measured with a moisture meter (Model GAC 2000, DICKEY-John Corp., Springfield, IL). Grain yield was corrected to 125 g kg<sup>-1</sup> moisture content. Nitrogen accumulated in both stover and grain, total N uptake, and N utilization efficiency (Moll et al., 1982) were calculated using the following equations:

$$\text{N accumulated in stover (kg ha}^{-1}\text{)} = \text{stover yield} \times (\text{N\% in stover}/100) \quad \text{Eq. [1]}$$

$$\text{N accumulated in grain (kg ha}^{-1}\text{)} = \text{grain yield} \times (\text{N\% in grain}/100) \quad \text{Eq. [2]}$$

$$\text{Total N uptake (kg ha}^{-1}\text{)} = \text{N accumulated in stover} + \text{N accumulated in grain} \quad \text{Eq. [3]}$$

$$\text{N utilization efficiency (kg kg}^{-1}\text{)} = \text{grain yield} / \text{total N uptake} \quad \text{Eq. [4]}$$



### *Soil nitrous oxide emission measurements*

Measurements of N<sub>2</sub>O were conducted using the closed static chamber method with polyvinylchloride (PVC) chambers, according to the USDA-ARS GRACEnet Project Protocols (Parkin and Venterea, 2010). The chamber consisted of two parts: a PVC ring anchor (30.3 cm diameter × 15 cm long) and a vented closed chamber head (30.3 cm inner diameter × 10 cm long) that was covered on the top and side with reflective Mylar Film tape.

The chamber anchors were inserted 10 cm into the soil, near the center rows of each plot, and left in place during the entire growing season. During the winter wheat and cover crop phases, the anchors were placed in the center of the plots, covering the entire space between rows. However, during the sorghum phase, the anchors were installed in the 4th row, such that a single row of both sorghum and N fertilizer band would be located in the center of the chamber. Measurements were generally made during 0900 h to 1300 h local time. On each sampling date, the vented PVC chamber head was placed on the anchor. Each chamber had an airtight septum at the top through which samples were collected. Individual gas samples of 20 mL were taken from the chamber at 0, 15, 30, and 45 min following chamber deployment using a 30-mL syringe, and immediately transferred into a 12-mL previously evacuated glass vial sealed with butyl rubber septum (Labco Ltd.). Gas samples were stored in the glass vials until analyzed by gas chromatography (Varian 450-GC) with an electron capture detector (ECD) (standard deviation of EDC = 0.009 µg L<sup>-1</sup>) to quantify N<sub>2</sub>O-N, as described by Wilson et al. (2015).

In general, gas flux measurements were made every other week from winter wheat planting to spring green-up in March, every other day after N fertilizer application (topdress) for 1 week, and then once a week until harvest. For the cover crop phase during 2014-15, the first gas flux measurements were done 42 days after planting, the second measurement 85 days after the first, and every other week thereafter. During the cover crop phase of 2015-16, gas flux measurements

were made every week from planting to termination of the summer CCs, and every other week thereafter. For the sorghum phase, gas flux measurements were made every other day during the first 10 days after N application, once a week for 60 days thereafter, and then every two weeks until harvest.

### ***Daily and cumulative nitrous oxide flux calculation***

Nitrous oxide fluxes were calculated from the rate of change of the concentration of the respective gas in the chamber headspace, as described by (Parkin and Venterea, 2010). Briefly, linear regression was used to calculate the slope of the N<sub>2</sub>O concentration [ppm(v)] vs. time, and the slope of the equation was assumed to be the N<sub>2</sub>O flux in  $\mu\text{L N}_2\text{O L}^{-1} \text{ min}^{-1}$ . Multiplying the slope by the chamber volume (L) and dividing by the chamber surface area (m<sup>2</sup>) resulted in a flux with units of  $\mu\text{L N}_2\text{O m}^{-2} \text{ min}^{-1}$ . The ideal gas law, the molecular weight of the N<sub>2</sub>O molecule (44 g mol<sup>-1</sup>), and the appropriate unit conversion were then used to adjust the flux to a mass per area-time base (g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>). The daily N<sub>2</sub>O flux for each treatment was considered to be the average of four replications of each treatment.

The cumulative area-scaled N<sub>2</sub>O emissions were estimated using trapezoidal integration of flux versus time, which in effect assumes that fluxes changed linearly between measurement dates, according to Ventera et al. (2011):

$$\text{Cumulative flux (kg N}_2\text{O ha}^{-1}) = \sum_{i=1}^n \left[ \frac{(X_i + X_{i+1})}{2} \right] \times (T_{i+1} - T_i) \quad \text{Eq [5]}$$

Where  $X_i$  is the initial N<sub>2</sub>O flux (kg N<sub>2</sub>O ha<sup>-1</sup> day<sup>-1</sup>), and  $X_{i+1}$  is the subsequent flux at times  $T_i$  and  $T_{i+1}$ , respectively.

### ***Yield-scaled nitrous oxide emissions and fertilizer induced emission factor***

Yield-scaled N<sub>2</sub>O emissions rates (YSNE) were calculated for the 2014 CCs through the 2015 grain sorghum harvest as an indicator of cropping system N<sub>2</sub>O efficiency, as proposed by Van Groenigen et al. (2010). Three different indexes of YSNE and the fertilizer induced emission factor (FIEF) were calculated according to the following equations:

$$\text{YSNE}^1 (\text{g N}_2\text{O kg}^{-1} \text{ N uptake}) = \text{Cf/Nt} \quad \text{Eq. [6]}$$

$$\text{YSNE}^2 (\text{g N}_2\text{O kg}^{-1} \text{ N in grain}) = \text{Cf/Ng} \quad \text{Eq. [7]}$$

$$\text{YSNE}^3 (\text{g N}_2\text{O Mg}^{-1} \text{ grain produced}) = \text{Cf/Gy} \quad \text{Eq. [8]}$$

$$\text{FIEF (\%)} = (\text{Cff} - \text{Cfu})/\text{Na} \quad \text{Eq. [9]}$$

where: Cf is the cumulative flux from cover crop planting through sorghum harvest; Nt is the total N uptake by aboveground sorghum biomass; Ng is the total N accumulated in sorghum grain; Gy is the sorghum grain yield; Cff is the cumulative flux of the fertilizer plot; Cfu is the cumulative flux of the unfertilized plot; and Na is the N fertilizer applied.

### ***Ancillary measurements***

At each gas sampling event, the soil water content (0-5 cm) was measured by taking one soil sample in the proximity of the chamber and oven drying at 105°C. Bulk density values were used together with gravimetric water content to estimate water-filled pore space (WFPS). The soil temperature was measured at 5-cm depth using a digital temperature probe. Air temperature inside the chamber was measured at each sampling time using a digital temperature probe. Measurements of precipitation were collected from a weather station located at the research farm (located approximately 20 m from the study site).

Soil samples for NO<sub>3</sub><sup>-</sup> and ammonium-N (NH<sub>4</sub><sup>+</sup>) determination were taken twice per month during the 2015-16 growing season for the winter wheat and cover crop phase. Composite soil

samples were collected at a 5-cm depth from each plot using a soil probe sampler with 2-cm diameter tube. Composite samples consisted of a total of eight individual cores per plot, randomly collected. The samples were dried in a forced-air oven at 40°C for 5 days, ground to pass a 2-mm screen using a soil grinder equipped with a ceramic mortar, metal screw type grinding head, and stainless steel screen (Nasco-Asplin, Fort Atkinson, WI), and analyzed for  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N using the K chloride (KCl) extraction method at the K-State Soil Testing Laboratory. Briefly, for soil-extractable  $\text{NO}_3^-$ -N, a 1 M KCl extraction (2g of soil in 20 mL extraction, shaken for 15 min) and cadmium reduction/colorimetry were used, according to Gelderman and Beegle (1998). Ammonium-N was extracted from soil samples with 1 M KCl (2g of soil in 20 mL extraction, shaken for 30 min) and measured by an indophenol colorimetric reaction as described by Keeney and Nelson (1982).

### *Statistical analysis*

Data were analyzed using a split-plot randomized complete block design, with cover crop as the whole plot factor and N rate as the sub-plot factor. Daily  $\text{N}_2\text{O}$  fluxes, and soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations were analyzed using PROC GLIMMIX in SAS<sup>®</sup> software (SAS Institute Inc, Cary, NC), with lognormal distribution to fulfill the assumptions of normal distribution and equality of variances. After calculating the ANOVA, the data were back-transformed to the original scale to aid interpretation. Cumulative  $\text{N}_2\text{O}$  flux, FIEF, and YSNE were analyzed by standard three-way ANOVA using PROC GLIMMIX, and year was included in the analysis and treated as a fixed effect to determine interactions involving year. Fisher's Least Significant Difference (LSD) method was used to assess the difference between the means of the treatments. Main effects and all interactions were considered significantly different at  $P \leq 0.05$ , unless noted otherwise. In addition, to evaluate the degree of association between  $\text{N}_2\text{O}$  emissions and soil and

weather variables, the data were submitted to analysis of correlation and multiple linear regression using PROC CORR and PROC REG. For the multiple regression analysis, the “stepwise” method was used with significance level of 0.05 for the inclusion of the variable into the model.

## **Results**

### ***Weather condition***

The cumulative precipitation in 2014 and 2015 were below and above the 30-yr average, respectively (Table 3.1). During the period of high precipitation (April to September), the study site received about 456 and 707 mm of rain in 2014 and 2015, respectively. In 2016, precipitation amounts in April and May were 214 and 177 compared to 81 and 129 mm for the 30-yr average, respectively. Very little precipitation occurred in January and December of 2014 and March of 2015 relative to the 30-yr average. Precipitation amounts during the sorghum growing season in 2015 equaled 436.6 mm, which is near the minimum required (457 mm) by sorghum plants to produce a normal yield.

### ***Winter wheat phase***

Daily N<sub>2</sub>O emissions during the winter wheat phases were significantly affected by the interaction of cover crop and N rate in 2014-15 growing season (Table 3.2). However, in the 2015-16 growing season, neither CCs, N rate, nor CCs × N rate influenced daily emissions (Table 3.2).

Despite differences in magnitude among cover crop treatments, the temporal behavior of N<sub>2</sub>O emissions followed a similar pattern in all N rate treatments (Fig 3.1a-c), with fluxes ranging from 0.35 to 34 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>. Except on 6 Dec 2014 and 16 Jan 2015, emissions were low and stable during 176 days after planting, and increased slightly thereafter until wheat harvest. In addition, the peak of N<sub>2</sub>O was measured, in all cover crop treatments, 1 week before harvest (17 June 2015), followed by an abrupt decrease at 1 day before harvest (25 June 2015). The greatest

fluxes were observed in plots historically grown with SS across N rates. In contrast, the ANOVA did not show any significant effect on daily N<sub>2</sub>O emissions during the 2015-16 wheat phase, perhaps reflecting the low variability among and within the treatments (Fig 3.2).

The three-way interaction of year  $\times$  cover crop  $\times$  N rate was not significant for cumulative N<sub>2</sub>O emissions (Table 3.3). However, the interaction of year and cover crop treatments was significant, therefore, the data were pooled across N rates. In general, cumulative N<sub>2</sub>O emissions from 2014-15 were significantly greater than from 2015-16 growing season for all legacy cover crop treatments (Fig 3.3). In 2014-15, SS had the greatest cumulative emission compared to DSB and DR, but it was not significantly different from CF. In the 2015-16 growing season, cumulative N<sub>2</sub>O emissions were not significantly different between cover crop treatments, ranging from 0.24 to 0.32 kg N<sub>2</sub>O-N ha<sup>-1</sup>.

### *Cover crop phase*

During the cover crop phases, there was no significant interaction between cover crop and N rate in the daily N<sub>2</sub>O emissions in either growing season (Table 3.2). Furthermore, N rate did not significantly influence on daily emissions; however, there was a significant difference among cover crop treatments.

In general, the overall pattern of N<sub>2</sub>O emissions was temporally similar in both years (Fig 3.4a and b). The initial pattern observed in 2014 was due to infrequent sampling (first sampling event occurring 5 weeks after cover crop planting, and the second sampling event 12 weeks thereafter). At the first sampling event, the CF treatment significantly increased N<sub>2</sub>O emissions (19.7 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>), whereas DSB, SS, and DR did not differ from each other (ranging from 2.86 to 4.98 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>) (Fig 3.4a). Fluxes of N<sub>2</sub>O were low and stable after the first frost and increased in early spring until sorghum was planted in 2015, reaching up to 14 g N<sub>2</sub>O-N ha<sup>-1</sup>

day<sup>-1</sup>. Double-crop soybean increased N<sub>2</sub>O emissions in two sampling event during the spring (on 7 Apr 2015 and 23 Apr 2015), followed by DR, CF, and SS.

A similar trend was observed in the 2015-16 growing season (Fig 3.4b). Overall, N<sub>2</sub>O fluxes were greater during the 3 weeks after cover crop planting for all treatments and decreased and remained at background levels thereafter, except CF. The highest emissions occurred 2 weeks after planting, in all treatments, ranging from 31 to 34 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>. Furthermore, the CF treatment had significantly greater N<sub>2</sub>O emissions from 3 weeks after CCs planting to close to the first frost day (from 16 July 2015 to 21 Oct 2015) when compared to the other cover crop treatments. During this period, the CCs and DSB reduced emissions by 65%, on average.

None of the interaction effects were significant for cumulative N<sub>2</sub>O emissions during the cover crop phase (Table 3.3). Only the main effects of cover crop and year significantly affected cumulative emissions. Across both growing seasons, CF increased the cumulative N<sub>2</sub>O emission by 2-fold when compared to the CCs and DSB, on average. In addition, no difference was observed in cumulative emissions among SS, DR, and DSB (Fig 3.5a). There were higher emissions in the 2015-16 than in the 2014-15 growing season for all fallow alternative treatments (Fig 3.5b).

The cover crop treatments also influenced soil NO<sub>3</sub><sup>-</sup> concentration during the fallow period of 2015-16 ( $P < 0.0001$ ), but did not affect NH<sub>4</sub><sup>+</sup> concentration ( $P = 0.7$ ). Within 2 weeks after planting, the two CCs and the DSB reduced soil NO<sub>3</sub><sup>-</sup> concentration by 20-fold when compared to the CF treatment, and the low levels of NO<sub>3</sub><sup>-</sup> persisted over the remainder of the fallow period (Fig 3.6). In general, soil NO<sub>3</sub><sup>-</sup> concentration tended to be low in the plots with CCs and DSB at least until the end of the winter, and increased during the spring.

During the period of the highest N<sub>2</sub>O emissions (from cover crop planting to first frost), soil moisture (expressed as WFPS), temperature, and NO<sub>3</sub><sup>-</sup> concentration were the variables that

best correlated with daily emissions, explaining about 68% of emission's variability ( $P < 0.0001$ ). When considering the entire fallow period, the regression analysis showed that WFPS, soil and air temperature were the variables that positively correlated with daily emissions, explaining 32% of the emission's variability ( $P < 0.0001$ ).

### ***Grain sorghum phase***

#### ***Daily and cumulative nitrous oxide emissions***

Daily  $\text{N}_2\text{O}$  emissions during the sorghum phase of the rotation were significantly influenced by the cover crop  $\times$  N rate interaction (Table 3.2). The effects of CCs on daily  $\text{N}_2\text{O}$  emissions were somewhat different in the 0-N plots (Fig 3.7a) in comparison to when N was applied (Fig 3.7b and c). Emissions from the SS cover crop plots that received no N fertilizer were predominantly lower throughout the sorghum growing season when compared to the other cover crop treatments (Fig 3.7a). Furthermore, the initial high flux events after sorghum planting ranged from 6 days on plots with previous SS to 60 days with CF.

Before UAN application,  $\text{N}_2\text{O}$  emissions were at background levels (less than  $10 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ ), and increased after the fertilizer application (Fig 3.7b and c). Four days after application of 28% UAN fertilizer,  $\text{N}_2\text{O}$  emissions increased significantly from background levels to  $280 \text{ (DSB plots)}$  and more than  $600 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$  (SS plots) in the 90-N and 180-N treatments, respectively. Additional distinct spikes in emissions were observed on 18 June 2015 and 22 July 2015 at the 180-N treatment. For all cover crop treatments, on average, the duration of the primary flux period after N fertilizer application was approximately 26 and 60 days for the 90-N and 180-N treatments, respectively; thereafter, emissions were reduced to background levels until sorghum harvesting, even after major precipitation events that occurred later in the season.



Total cumulative N<sub>2</sub>O emissions were affected by cover crop and N rate, but with no significant interaction (Table 3.3). At the end of the grain sorghum phase, when averaged across cover crop treatments, increasing N rate significantly increased total cumulative flux (Fig 3.8a). Cover crop treatments also affected cumulative emission, with greater emissions in the DSB and SS treatments than in the DR and CF (Fig 3.8b).

#### ***Yield parameters and nitrogen utilization efficiency***

The interaction between cover crop and N rate was significant for grain yield ( $P = 0.0004$ ). Maximum grain yield occurred when N was applied at a rate of 90 kg ha<sup>-1</sup> for sorghum grown after CF (Fig 3.9a), 135 kg ha<sup>-1</sup> after DR (Fig 3.9d), and 180 kg ha<sup>-1</sup> after DSB and SS (Fig 3.9b and c, respectively). However, no significant yield difference was observed for the CF, DR, and DSB treatments when the N fertilizer rate increased from 90-N to 180-N. Similarly, no yield difference was observed for sorghum following the SS cover crop when the N fertilizer rate increased from 135-N to 180-N.

Dry matter yield, total aboveground N uptake, and grain N yield were affected by cover crop and N rate, but with no significant interaction (Table 3.4). Dry matter yield was greater in sorghum grown after DSB, DR, and CF than after SS. Chemical fallow and DSB had the greatest total N uptake and grain N yield, followed by DR and SS. When averaged across cover crop treatments, increasing N rates significantly increased DM, total N uptake, and grain N yield.

Nitrogen utilization efficiency was affected by the interaction of cover crop and N rate ( $P = 0.04$ ). Overall, N utilization efficiency decreased with increasing N fertilizer rate in all treatments, except SS (Fig 3.10). Sorghum-sudangrass and DR had greater N utilization efficiency at 90-N when compared to CF. At 180-N, N utilization efficiency was greatest for the SS plots when compared to the other cover crop treatments.

### ***Cropping system cumulative and yield-scaled nitrous oxide emissions, and fertilizer induced emission factor***

When evaluating the entire cropping system period of the 2014 CCs through the 2015 grain sorghum harvest, total cumulative N<sub>2</sub>O emissions were not affected by the interaction of cover crop and N rate, or by cover crop treatments; however, N rate significantly influenced cumulative emissions (Fig 3.11a and b; Table 3.5). Overall, increasing N rate during the sorghum phase increased total emissions of the cropping system (Fig 3.11b; Table 3.5).

Nitrous oxide emissions scaled by grain yield, grain N yield, and total N uptake were all affected by N rates (Table 3.6). The overall N rate effects were similar when emissions were expressed per unit of grain and grain N yield, with lower emissions for the 0-N control, and no significant difference between the remaining N rates. When scaled by total N uptake, the 90-N emissions (25.7 g N<sub>2</sub>O-N kg<sup>-1</sup> aboveground N uptake) were not different from the 0-N control (18.5 g N<sub>2</sub>O-N kg<sup>-1</sup> aboveground N uptake) or the 180-N treatment (28.6 g N<sub>2</sub>O-N kg<sup>-1</sup> aboveground N uptake).

Emissions did not vary by cover crop when scaled by grain yield and grain N yield across N rate treatments, but the difference in emissions scaled by total N uptake were only significant at the  $P \leq 0.1$  confidence level (Table 3.6). When averaged across N rates, emissions were greater in plots that had the SS cover crop, whereas no difference was observed among the remaining treatments.

Fallow management was the only factor that had a significant effect on FIEF (Table 3.6). Across N rates, DR significantly reduced FIEF when compared to DSB and SS, but it was similar to CF. Because there was no value of FIEF for the 0-N control treatment, regression analysis of FIEF vs. N rate did not generate significant models.

Emissions of  $\text{N}_2\text{O}$  were linearly correlated with fertilizer N rate for all cover crop treatments (Fig 3.12a). However, the magnitude of the relationship differed among the treatments, with two distinct trends. The slopes of the regression equations for CF and DR were 9.4 and 11.2 g  $\text{N}_2\text{O-N kg}^{-1}$  N applied, respectively, but for DSB and SS regression slopes were 27 and 33.6 g  $\text{N}_2\text{O-N kg}^{-1}$  N applied, respectively. Similar trends were observed when emissions were scaled per unit grain and plotted against N fertilizer rate (Fig 3.12b). For sorghum grown after DSB and SS, YSNE increased as fertilizer N rate increased at rates of 3.18 and 2.50 g  $\text{N}_2\text{O-N per Mg}$  of grain produced, respectively, in contrast to 0.70 and 1.06 g  $\text{N}_2\text{O-N Mg}^{-1}$  grain for sorghum grown after CF and DR, respectively.

Regression analysis between total cumulative  $\text{N}_2\text{O}$  emissions and N utilization efficiency showed that, for all cover crop treatments, emissions decreased with increasing N utilization efficiency (Fig 3.13a). When emissions were scaled per unit N uptake and plotted against N utilization efficiency, the CF was the only treatment that increased emissions with increase N utilization efficiency (Fig 3.13b).

## Discussion

### *Residual effects of cover crops on the winter wheat phase of the crop rotation*

The pattern of  $\text{N}_2\text{O}$  emissions during the wheat phase of the rotation were, in general, different from that during the cover crop and grain sorghum phase. During the wheat phase, the spikes of emissions occurred towards the end of the growing season, and they were likely related to the increased soil mineral N content after N top-dress and the combination of warm and moist soil, with WFPS > 65% (data not shown). It is well documented that soil moisture is a major driver of  $\text{N}_2\text{O}$  emissions as it regulates the oxygen availability to soil microorganisms (Butterbach-Bahl et al., 2013). During the denitrification process, large nitric oxide (NO) emissions can be expected

at WFPS values of 30 to 60%, and large N<sub>2</sub>O emissions when WFPS ranges from 50 to 80%; whereas under wetter soil conditions (above 80% WFPS), N<sub>2</sub> should be the dominant gas emitted, depending on soil type (Mosier et al., 2002). This explains why emissions persisted at background levels even after N fertilizer application soon after spring greenup. Although the soil was relatively warm after N top-dress (> 15°C), the low emissions were mainly due to a dry period that lasted from 2 Feb to 1 Mar 2015 (with 3 precipitation events of less than 3 mm), resulting in WFPS < 40% (data not shown). At 1 week before harvest, the wheat plants had already reached physiological maturity, and the residual N (from the N top-dress and organic matter mineralization) coupled with rainfall events may have favored denitrification-driven N<sub>2</sub>O emission. Emissions of N<sub>2</sub>O in winter wheat in Oklahoma (Wilson et al., 2015), Montana (Dusenbury et al., 2008), and Canada (Drury et al., 2008) followed a similar pattern as that found in this study, with low emissions during the winter and an increase in emissions during late spring/early summer.

Crop rotation adds another dimension that is often overlooked because the crop residue being decomposed and supplying soluble C to soil biota is usually from a different crop than the crop that is currently growing (Drury et al., 2008). In this study, the cover crop treatments had different effect on cumulative N<sub>2</sub>O during the two years, showing significant and no significant differences in the 2014-15 and 2015-16 growing seasons, respectively. For this reason, it is difficult to evaluate the legacy effects from the past crop rotation and cover crop use.

Based on relevant literature to date, it is expected that the plots managed with high C:N ratio biomass cover crop (such as SS) will result in higher denitrification and N<sub>2</sub>O emissions rates because of higher soil organic C and microbial populations near the soil surface (Aulakh et al., 1992; Linn and Doran, 1984; Omonode et al., 2011), but because we did not measure those

parameters in this study, further analysis is needed to assess the specific effect of available C on N<sub>2</sub>O emissions during the wheat phase. In addition, it must be noted that in this cropping system, the winter wheat is planted about 2 years after the prior cover crop termination and DSB harvest. This period is likely long enough to decompose most of the cover crop residues on the soil surface, even the large amount of residues left from the SS. Moreover, the same N fertilizer rate was applied to all plots at the same time during the wheat phase (starter and top-dress). Therefore, it is expected that differences in N<sub>2</sub>O emissions were more directly related to differences in weather and precipitation patterns than the residual effects of the cover crop treatments; thus, the results of this study do not provide enough evidence that CCs are significantly affecting N<sub>2</sub>O emissions 2 years following the use of CCs in the crop rotation.

#### ***Role of cover crops on reducing nitrogen losses in the fallow period***

Although few measurements were taken within the first few months after cover crop planting in 2014, we can observe that the pattern of emissions was similar to the 2015-16 growing season, with primary flux periods occurring during the summer, low emissions during the winter, and a slight increase in emissions during late spring/early summer. The period of time with the largest fluxes lasted three weeks after cover crop planting in 2015 in all treatments, which might be related to the presence of residual mineral N from the previous wheat crop, coupled with high soil temperature, and precipitation events that favored microbial activity. Post-harvest soil N (residual fertilizer and mineralization) is a concern regarding N management because it is susceptible to losses through leaching and/or denitrification (Scharf, 2015). A plausible explanation for the greater emissions in the CF was that the lack of a cover crop resulted in increased residual profile N that was subject to loss as N<sub>2</sub>O (Fig 3.4a, 3.5). This would be in accordance with the significantly greater soil NO<sub>3</sub><sup>-</sup> concentration measured in the CF plots

throughout the fallow period (Fig 3.6). In addition, the positive correlation between WFPS, soil temperature and  $\text{NO}_3^-$  content, and  $\text{N}_2\text{O}$  fluxes indicates that denitrification played an important role in  $\text{N}_2\text{O}$  emissions during the summer, but denitrification-driven  $\text{N}_2\text{O}$  emissions were negligible during freeze-thaw cycles over the winter. Many studies have shown that CCs are very effective in taking up mineral N from soil that otherwise would be readily available for denitrification, especially during times of the year when soil organic matter decomposition and N mineralization are active (Baligar and Fageria, 2007; Kramberger et al., 2009; Tonitto et al., 2006). These results support claims that CCs or double-crop have the potential to reduce  $\text{N}_2\text{O}$  emissions during the fallow period after wheat harvest and retain N in the system for the subsequent crop.

### ***Nitrous oxide emissions during the grain sorghum phase***

It is well accepted that N fertilization is the major factor controlling  $\text{N}_2\text{O}$  emissions in agricultural soils (Snyder et al., 2009), and emissions of  $\text{N}_2\text{O}$  have been found to increase linearly or non-linearly with N fertilizer rate (Kim et al., 2013; Shcherbak et al., 2014). In our study, greater magnitude of  $\text{N}_2\text{O}$  emissions were seen as N rate increased, particularly within 2 months after N fertilizer application; thereafter, emissions returned to background levels until grain harvest. Although we did not measure soil mineral N for this period, the most prevalent hypothesis in the relevant literature to date is that the soil N concentration rapidly increased after N fertilizer application, but soil microorganisms and sorghum plants were not able to fully absorb plant available N, and therefore, the N surplus coupled with precipitation events were the main factors affecting  $\text{N}_2\text{O}$  emissions. In contrast, large precipitation events towards the end of the growing season were not enough to trigger  $\text{N}_2\text{O}$  emissions, probably due to low available N in the soil. Despite differences in weather and soil conditions, similar results were reported by Schwenke and Haigh (2016) on soil  $\text{N}_2\text{O}$  emissions in sorghum grown in Australia. The authors concluded that

later in the growing season, the sorghum crop had taken up most of the available N from the soil, thereby reducing the probability of significant differences in N<sub>2</sub>O fluxes.

The greater emissions following UAN application on plots that had previously contained SS and DSB could be attributed to the combination of many factors such as cover crop residue, fertilizer placement, rainfall, and soil moisture (WFPS). A precipitation event of 12 and 30 mm that occurred two days before the first peak of emissions (4 days after N fertilizer application) increased WFPS to 65%. It is known that the addition of legume residues to the soil enhances N<sub>2</sub>O emissions by increasing plant residue and organic matter mineralization rates, and the resulting increase in mineral N (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) and available C contents provides substrate for denitrification (Gomes et al., 2009). On the other hand, non-legume cover crop residues have the potential to decrease N<sub>2</sub>O emissions by decreasing soil mineral N through uptake during growth and microbial immobilization during residue decomposition (McSwiney et al., 2010). However, the injection of the liquid fertilizer below the SS residue layer may have reduced N immobilization at least until the spikes of N<sub>2</sub>O emissions were observed. Moreover, the SS residue layer conserved soil moisture, prolonging the period of high WFPS, and therefore, providing favorable conditions (anaerobic microsites) for denitrification process.

Regardless of the cover crop treatment, approximately 50% of the total N<sub>2</sub>O emissions occurred within 2 weeks after sorghum planting and N fertilizer application, suggesting that this period has the greater susceptibility to N losses through denitrification. Several studies also have reported increased N<sub>2</sub>O emissions directly after N fertilizer application. In a three-year corn study in Iowa, Parkin and Kaspar (2006) found that N<sub>2</sub>O emissions peaked at 650 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> in response to rainfall soon after N fertilizer application of 200 kg N ha<sup>-1</sup>. According to the authors, those peaks represented 49% of the total cumulative N<sub>2</sub>O emissions. Omonode et al. (2011) found

that 50% of total N<sub>2</sub>O emissions occurred shortly after N fertilizer application with similar N fertilizer rates in corn grown in Indiana. When averaged across cover crop treatments, the cumulative N<sub>2</sub>O emissions at the two N fertilizer rates in this study were similar in magnitude to those reported by Schwenke and Haigh (2016) in sorghum grown on sub-tropical soils in Australia, with annual N<sub>2</sub>O emissions ranging from 2.8 to 4.4 kg N<sub>2</sub>O-N ha<sup>-1</sup>. Across N rates, DSB and SS increased total N<sub>2</sub>O emissions by 65% when compared to CF and DR. Similar results were reported by Mitchell et al. (2013). The authors found an increase of about 15% for cumulative N<sub>2</sub>O emissions after fertilized corn following rye compared to CF.

### ***Cropping system effect on nitrous oxide emissions***

Our results showed that CCs or DSB were very effective at reducing N losses during the fallow period after winter wheat, but these effects did not persist throughout the grain sorghum phase (Table 3.5). These results indicate that while CCs have the potential to reduce N<sub>2</sub>O emissions, N fertilizer application might be the overriding factor as proposed by Jarecki et al. (2009). Although the range of the soil N<sub>2</sub>O emissions were greater in our study, similar effects of legume CCs preceding corn in southern Brazil were observed by Gomes et al. (2009). The authors found greater cumulative N<sub>2</sub>O emissions in a crop rotation of corn with pigeon pea, lablab, and vetch than with oat. Baggs et al. (2003) also reported that greater cumulative N<sub>2</sub>O emissions were measured from corn with cover crop residues than the CF control. In contrast to our study, Mitchell et al. (2013) found a significant interaction of cover crop × N rate in a no-till corn-soybean study in Iowa. The authors reported that winter rye decreased N<sub>2</sub>O emissions from corn when no N fertilizer was applied, but it increased when N was applied.

Increasing N fertilizer rate on the sorghum phase increased N<sub>2</sub>O emissions linearly ( $R^2 = 0.99$ ). The slope of the linear regression between N fertilizer rate and cumulative N<sub>2</sub>O emission



was 18.8 g N<sub>2</sub>O-N kg<sup>-1</sup> N applied, on average. Other authors have reported slopes ranging from 4.1 to 24.8 g N<sub>2</sub>O-N kg<sup>-1</sup> N applied on regression equations of N fertilizer input on sorghum and corn (Schwenke and Haigh, 2016; Venterea and Coulter, 2015). Recent literature has shown a majority of non-linear (exponential or hyperbolic) responses for N<sub>2</sub>O fluxes as a function of N fertilizer input, especially when N rate exceeded crop N requirement for maximum economic yield (Bouwman et al., 2002; Kim et al., 2013; Shcherbak et al., 2014); however, Kim et al. (2013) documented four published instances with linear responses to at least four N rate levels. In addition, the greater slopes for the regression between N fertilizer rate and cumulative N<sub>2</sub>O emissions for DSB and SS (Fig 3.12a) resulted in greater FIEF (Table 3.6). Our results revealed a larger range of FIEF (0.98 – 3.22%) compared with those (1 – 1.2 %) calculated by Bouwman et al.(2002) and the IPCC (2007).

Although N<sub>2</sub>O emissions are expected to increase with addition of low C:N ratio crop residues and N fertilizer rates, expressing emissions in relation to crop productivity (yield-scaled N<sub>2</sub>O emissions) may be more informative in both assessing N<sub>2</sub>O efficiency of a cropping system (Van Groenigen et al., 2010) and developing N management strategies to achieve optimum crop productivity and minimize environmental impacts (Schwenke and Haigh, 2016). Total N<sub>2</sub>O emissions and YSNE showed a significant relationship with N utilization efficiency (Fig 3.13a and b). Despite the differences in magnitude, increasing N utilization efficiency decreased N<sub>2</sub>O emissions for all cover crop treatments. The greater slopes for DSB and SS may indicate greater potential for N<sub>2</sub>O mitigation. For instance, when N utilization efficiency increased 1.5-fold, the N<sub>2</sub>O emissions decreased more than 2-fold in plots that with DSB compared to the CF treatment (Fig 3.13a). In addition, our results showed that the CCs and DSB might be improving N use efficiency when N is applied near the economic optimum rate of 90-N (Fig 3.10). This supports

Van Groenigen et al.'s (2010) conclusion that crop management practices that aim to increase fertilizer N use efficiency are directly related to those practices that aim to minimize N<sub>2</sub>O fluxes.

In summary, growing CCs in rotation with cash crops can reduce N<sub>2</sub>O emissions by taking up residual N during the fallow period, but they also can increase N<sub>2</sub>O emissions during the following cash crop. However, the effects of CCs on factors that influence N<sub>2</sub>O emissions are complex and interact with other management practices, such as residue management, N fertilizer rate, and N placement, which can be overriding factors (Jarecki et al., 2009; Mitchell et al., 2013). The potential impacts of greater soil N<sub>2</sub>O emissions from the inclusion of CCs need to be evaluated and weighed against the other benefits of CCs, such as reduced soil erosion, reduced NO<sub>3</sub><sup>-</sup> leaching and improved water quality, improved soil quality and health, as well as supplying N and improving crop yield (Steenwerth and Belina, 2008).

## **Conclusion**

Although the CCs and DSB affected total cumulative N<sub>2</sub>O emissions during the winter wheat phase of the rotation in only one out of two years, the results of this study suggest that differences in N<sub>2</sub>O emissions during the wheat phase were more likely related to the difference in weather conditions, with greater probability of emissions to occur in wetter years. An additional year of data collection is necessary to provide a more complete picture of the legacy effect of previous fallow and N management over time and solidify initial conclusions.

The results of this study support our initial hypothesis that the use of CCs would reduce N<sub>2</sub>O emissions during the fallow period. When considering the N dynamics throughout the cropping system, the use of CCs or DSB after winter wheat was confirmed to be a good strategy to reduce N losses (potential NO<sub>3</sub><sup>-</sup> leaching and N<sub>2</sub>O emissions) during the fallow period, especially during the summer when residual N accumulation and N<sub>2</sub>O emissions are expected to

be high. The low soil  $\text{NO}_3^-$  concentration, which was related to the N uptake by the CCs and DSB, explained the lower fluxes observed throughout the fallow period in comparison to CF.

The use of synthetic N fertilizer in the following grain sorghum crop tended to offset the legacy effects of CCs, with no significant difference between cover crop treatments for cumulative  $\text{N}_2\text{O}$  emissions at the end of the sorghum phase. These results reject our initial hypothesis that the legacy effect of CCs would reduce the cumulative emissions. Regardless of the cover crop treatment, about 50% of the total emissions occurred within 3 weeks after N application. This result emphasizes the importance of implementing N management strategies to reduce  $\text{N}_2\text{O}$  emissions early in the growing season, regardless of fallow management strategy.

Although sorghum grain yield was influenced by previous cover crop and N rate, the combination of these two treatments did not affect the overall  $\text{N}_2\text{O}$  efficiency of the cropping system, suggesting that the potential increase of  $\text{N}_2\text{O}$  emissions during the fallow period (for the CF treatment) and sorghum phase (DSB treatment) are being balanced with improved crop yield. This conclusion is based on the increased dry matter production, total N uptake, and N accumulated in sorghum grain following CF and DSB compared to SS and DR. Overall, SS was the only cover crop that significantly increased YSNE. A second year of data from the 2016 sorghum phase will provide a better understanding of the  $\text{N}_2\text{O}$  efficiency of the cropping system.

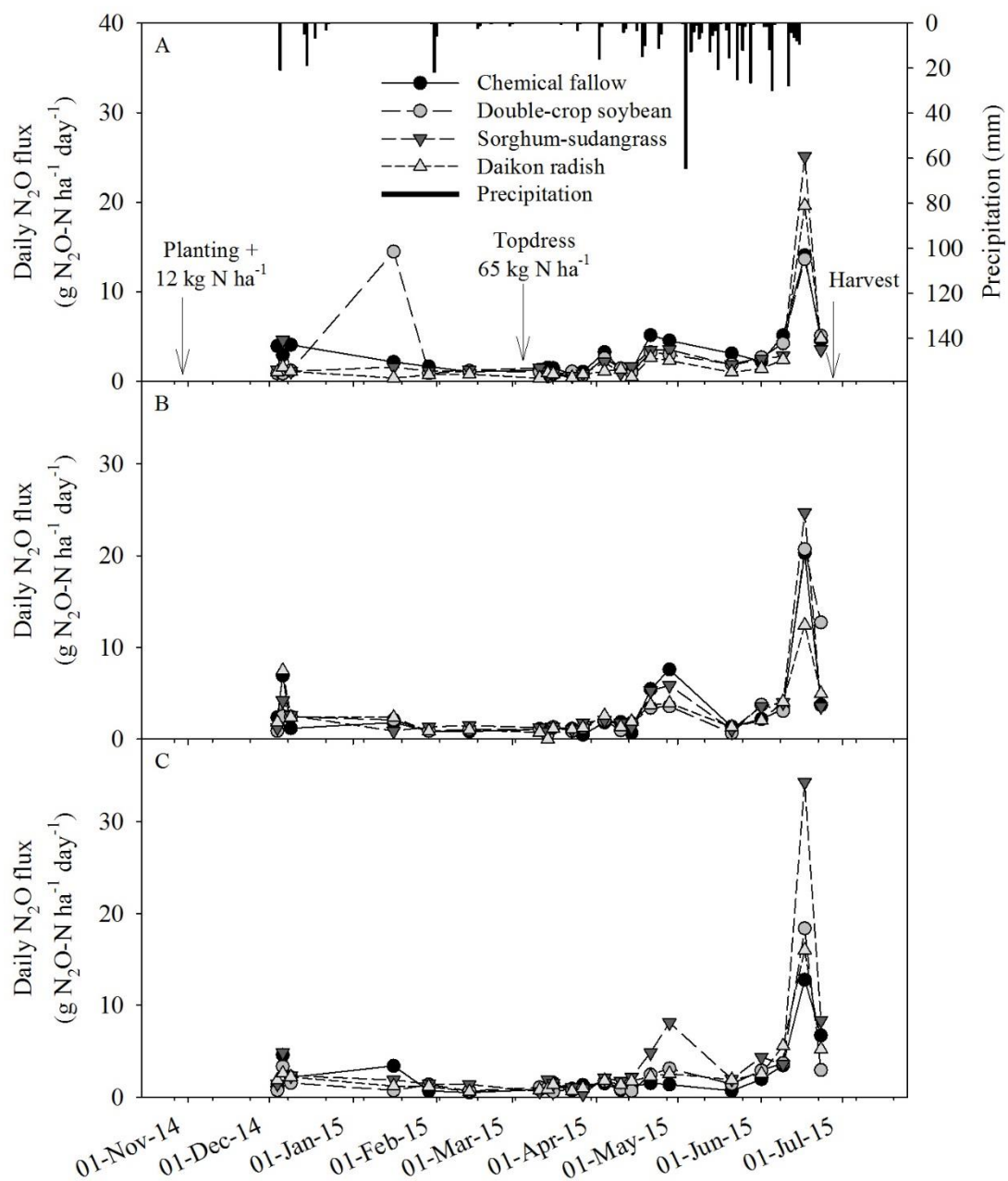
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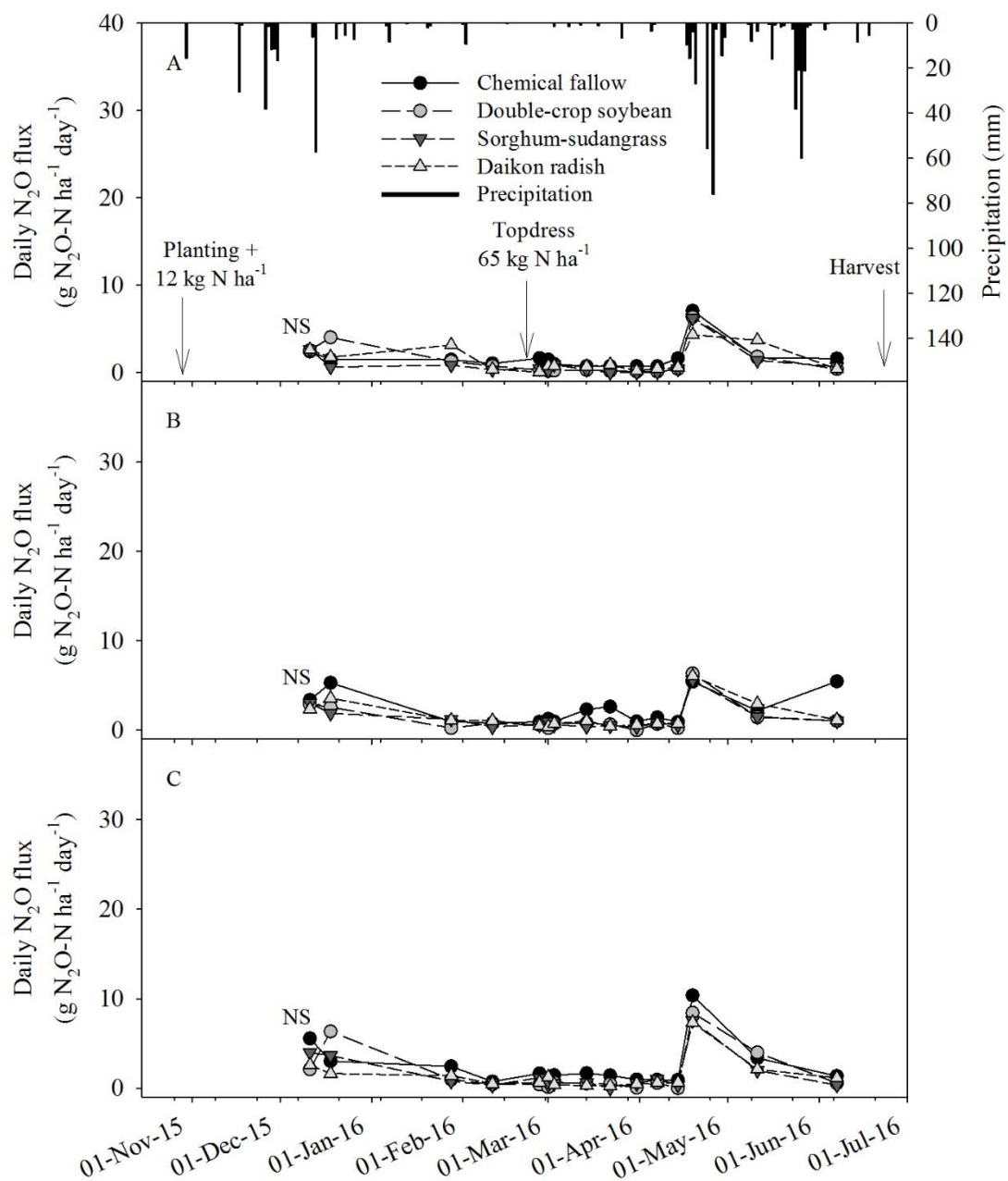
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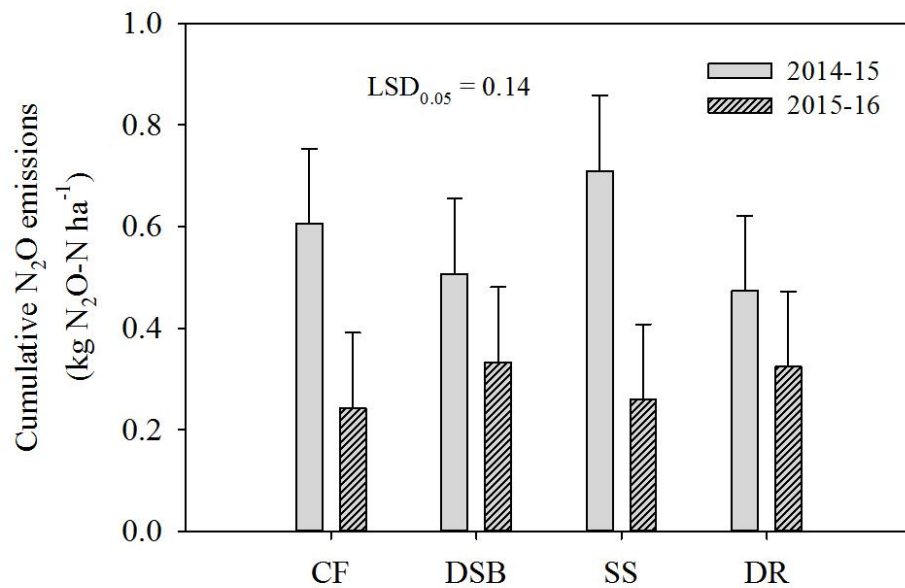


**Figure 3.1.** Mean daily nitrous oxide ( $N_2O$ ) emissions during the 2014-15 winter wheat phase of the rotation under different fallow and nitrogen (N) management. (A, 0 kg N  $ha^{-1}$ ; B, 90 kg N  $ha^{-1}$ ; C, 180 kg N  $ha^{-1}$  applied during the 2012 sorghum phase of the crop rotation. Previous cover crops and double-crop soybean were grown after wheat harvest in 2011.).

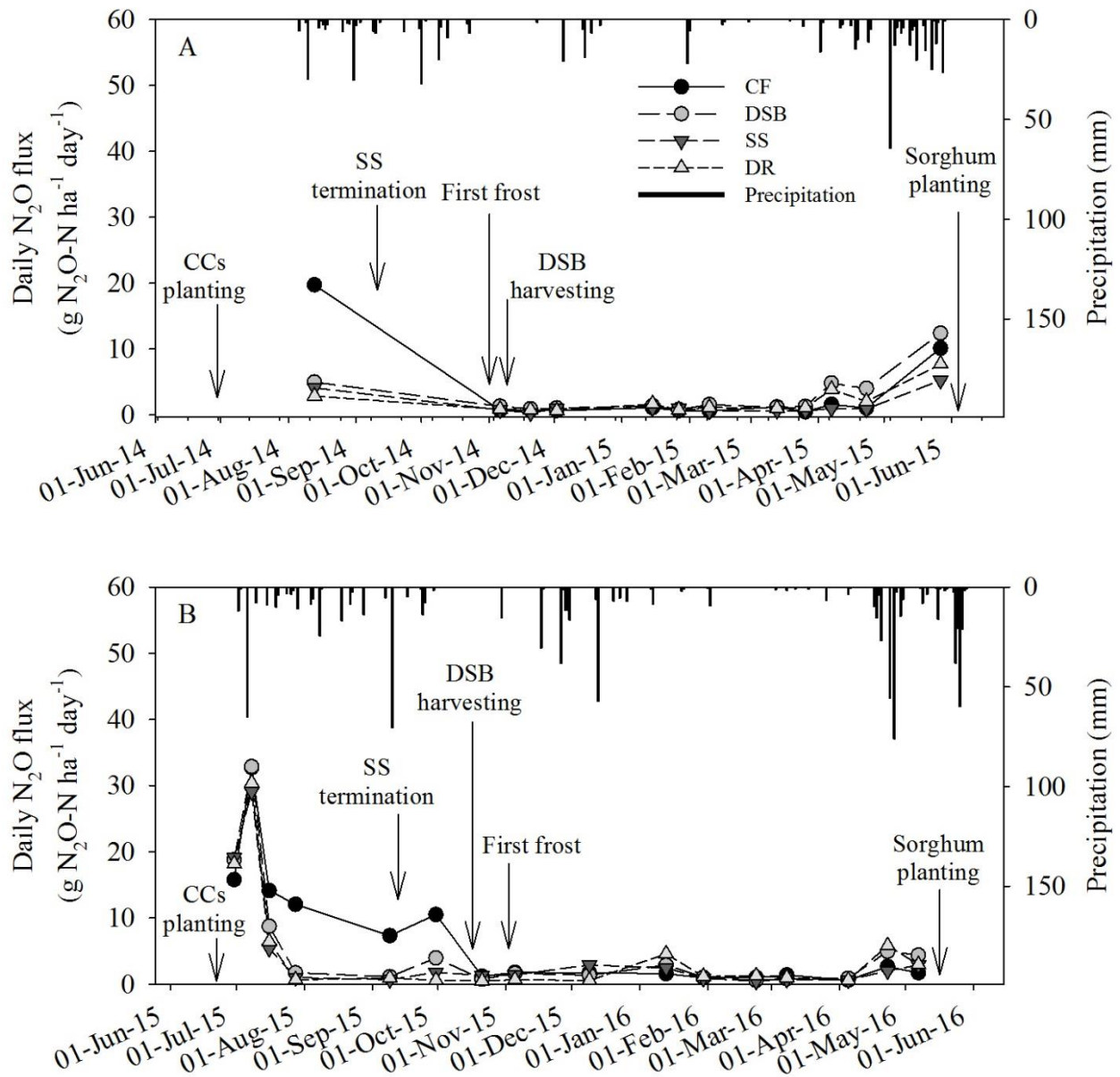




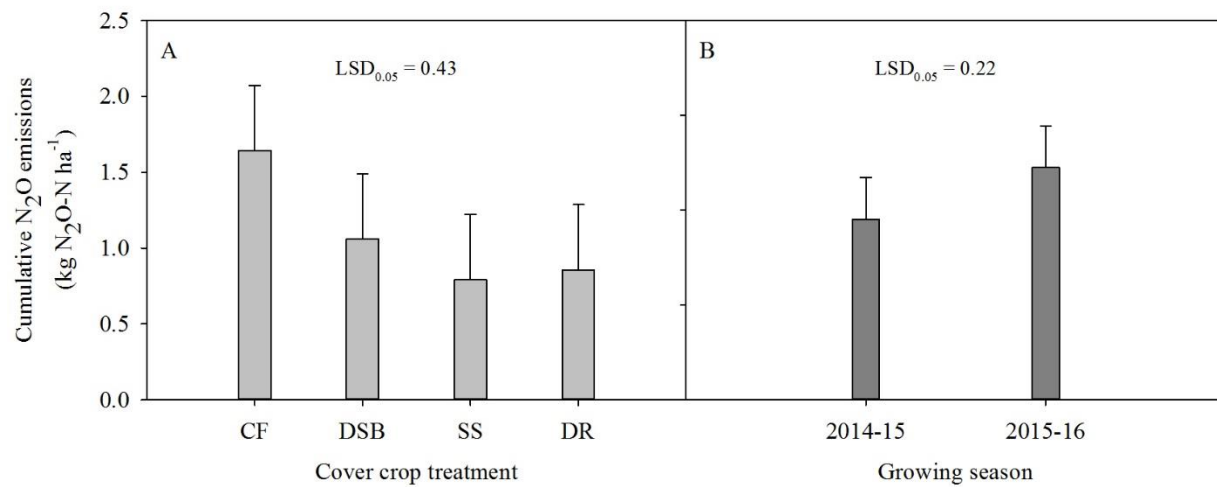
**Figure 3.2.** Mean daily nitrous oxide (N<sub>2</sub>O) emissions during the 2015-16 winter wheat of the rotation under different fallow and nitrogen (N) management. (A, 0 kg N ha<sup>-1</sup>; B, 90 kg N ha<sup>-1</sup>; C, 180 kg N ha<sup>-1</sup> applied during the 2013 sorghum phase of the crop rotation. Previous cover crops and double-crop soybean were grown after wheat harvest in 2012. NS, not significant.).



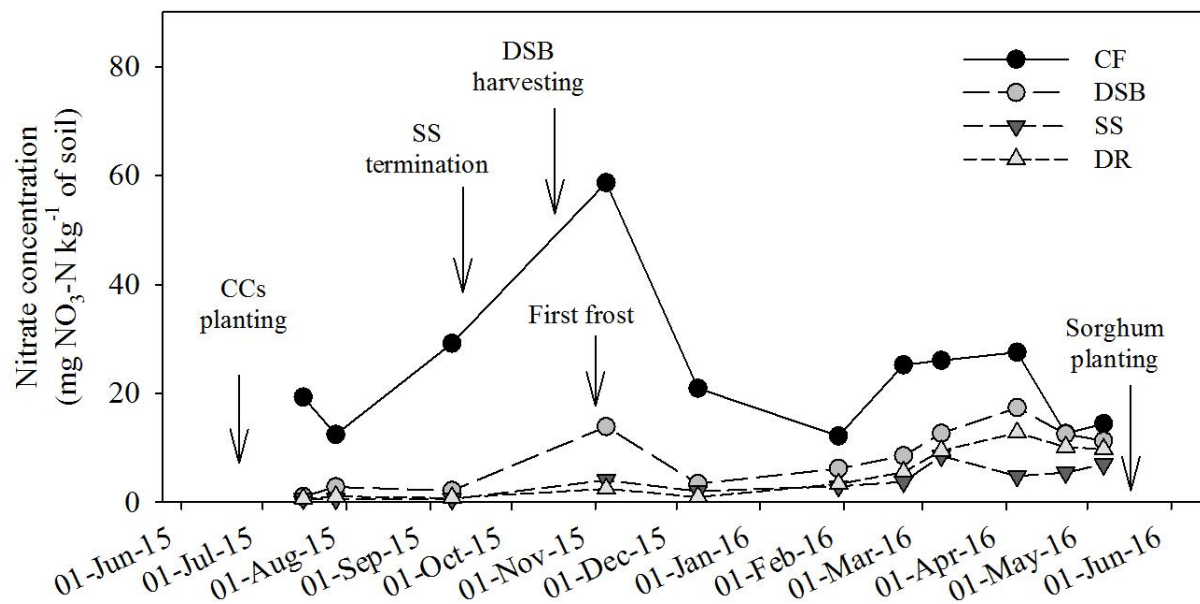
**Figure 3.3.** Residual effects of fallow management on total cumulative N<sub>2</sub>O emissions from winter wheat planting to harvest in the 2014-15 and 2015-16 winter wheat phase of the rotation at Ashland Bottoms, KS. Vertical bars indicate Fisher's LSD at  $P \leq 0.05$ . (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).



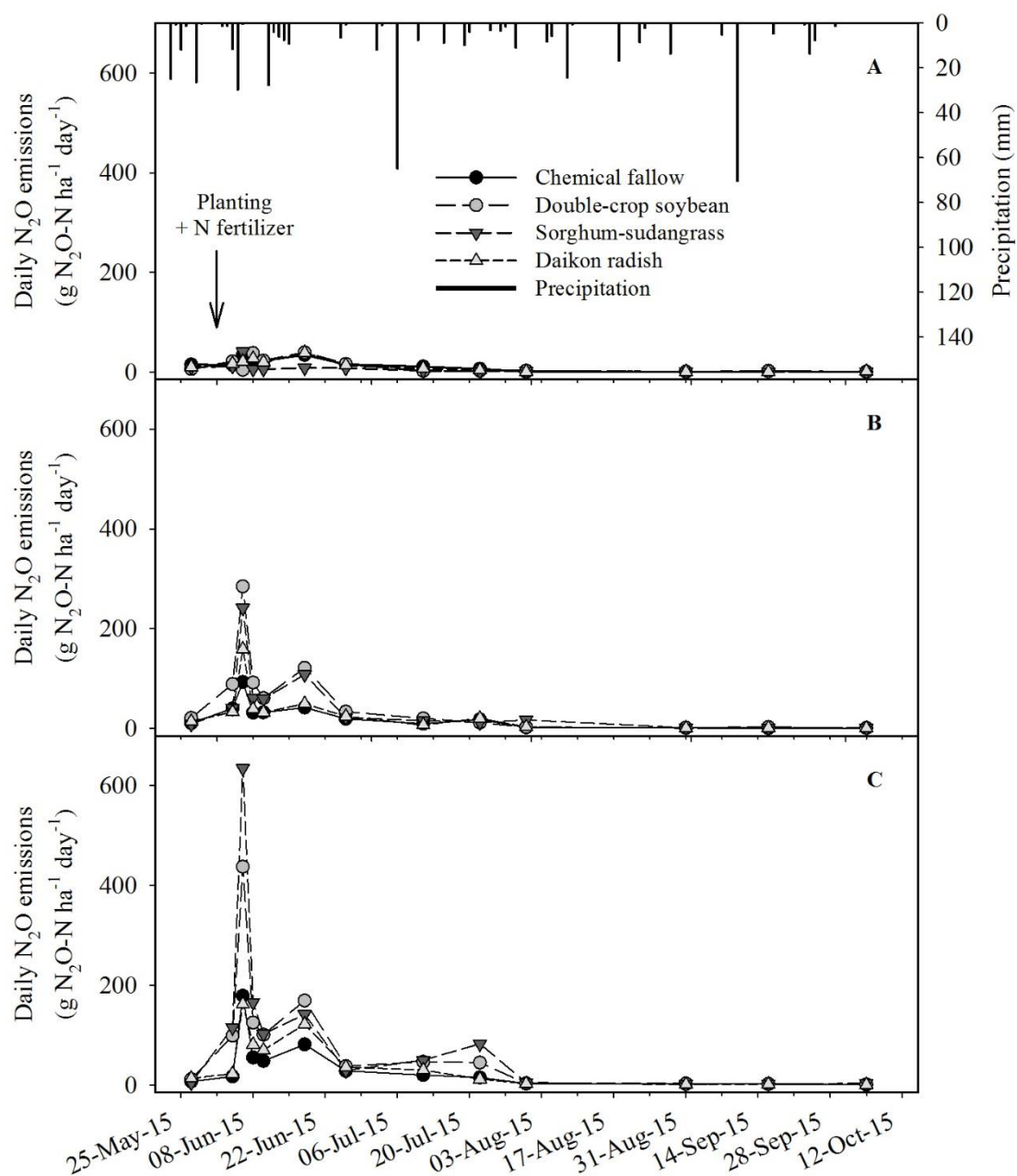
**Figure 3.4.** Mean daily nitrous oxide ( $N_2O$ ) emissions under different cover crop treatments during the 2014-15 (A) and 2015-16 (B) fallow period of the crop rotation at Ashland Bottoms, KS. (CCs, cover crops; CF, chemical fallow; DS, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish. First frost was the first day that minimum temperature was below 0°C.).



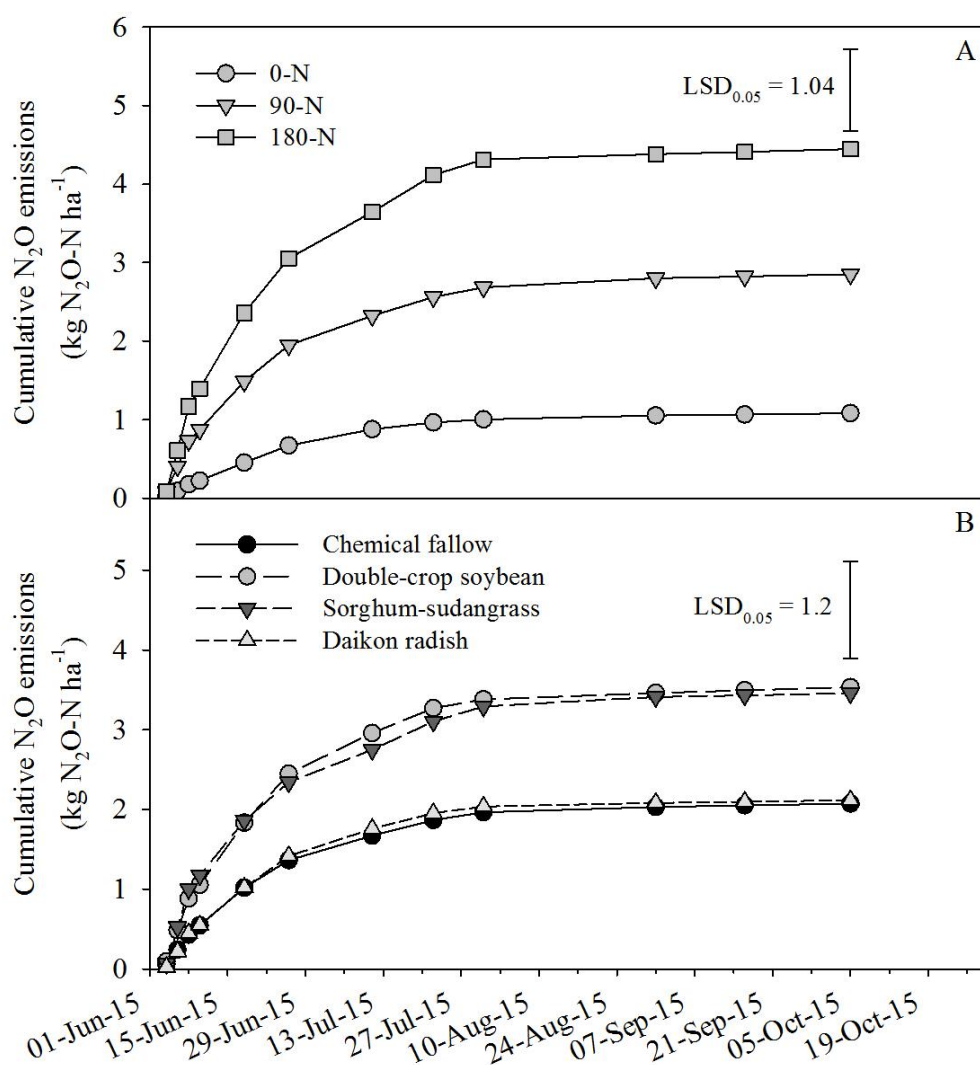
**Figure 3.5.** Total cumulative nitrous oxide ( $N_2O$ ) emissions as affected by cover crop treatments (A) and growing season (B) at the end of the fallow period of the rotation at Ashland Bottoms, KS. Vertical bars indicate Fisher's LSD at  $P \leq 0.05$ . (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).



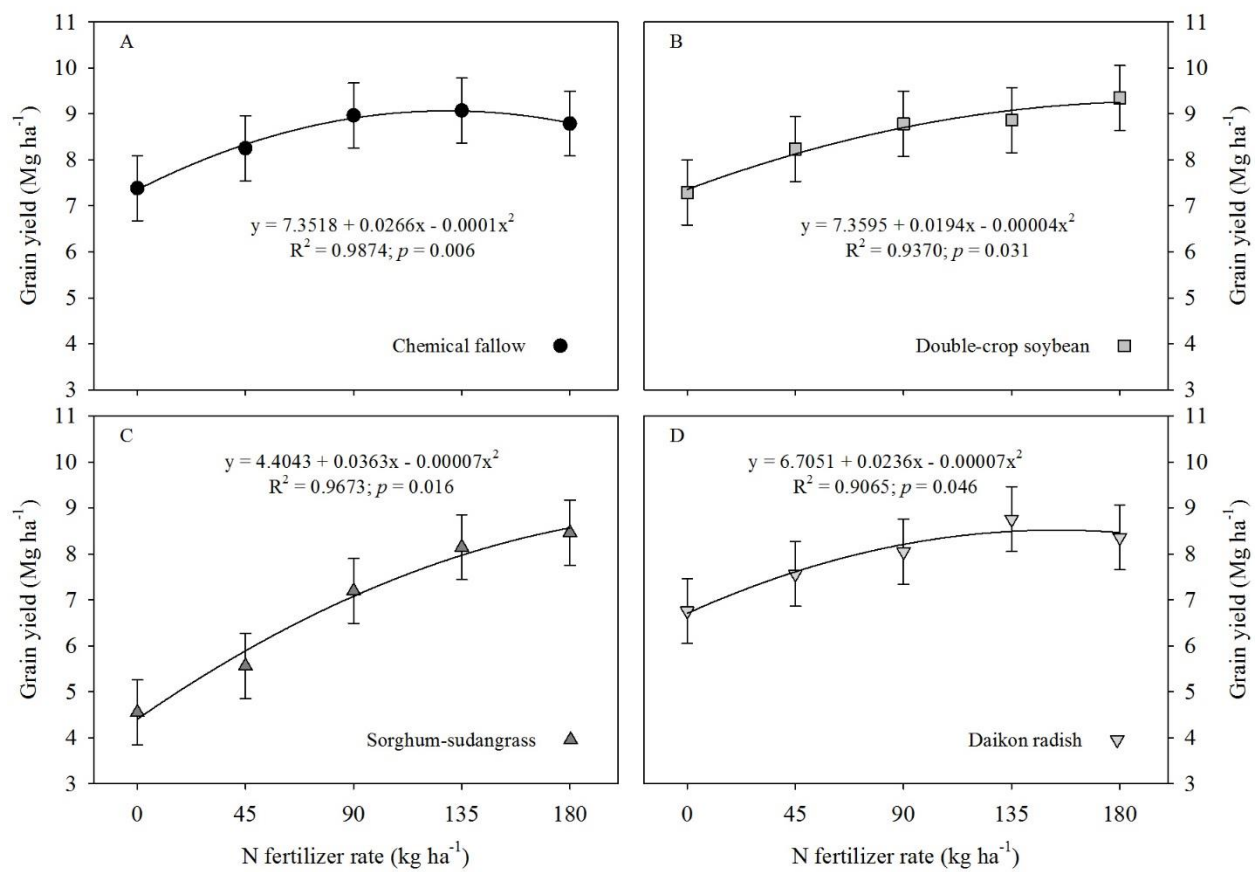
**Figure 3.6.** Soil nitrate concentration at the 0-5 cm depth in plots under different fallow management during the fallow period of 2015-16. (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).



**Figure 3.7.** Mean soil nitrous oxide ( $N_2O$ ) emissions as affected by different fallow management and nitrogen (N) rates during the 2015 grain sorghum phase of the rotation at Ashland Bottoms, KS. (A; 0 kg N  $ha^{-1}$ ; B, 90 kg N  $ha^{-1}$ ; C, 180 kg N  $ha^{-1}$ ; N fertilizer application with 28% Urea Ammonium Nitrate.).

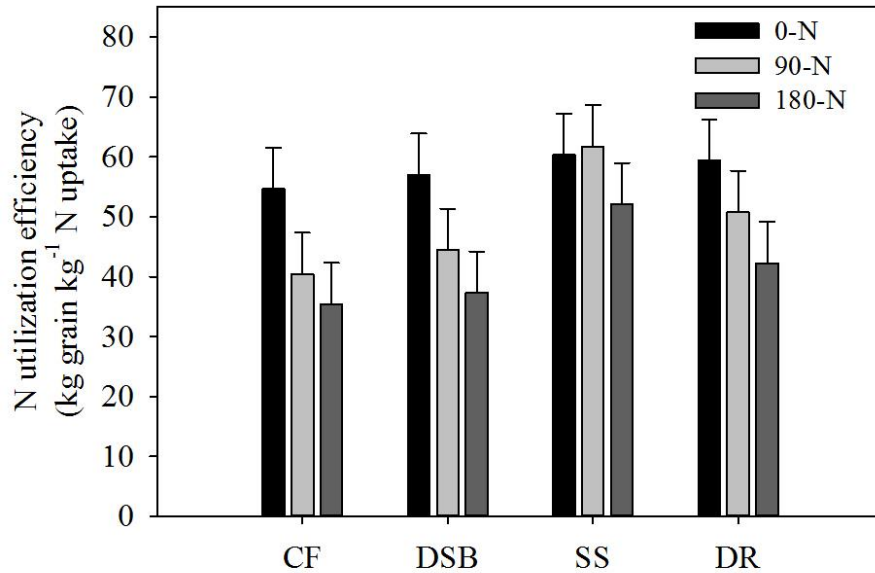


**Figure 3.8.** Mean total cumulative nitrous oxide (N<sub>2</sub>O) emissions during the grain sorghum growing season in 2015 as affected by nitrogen (N) fertilizer rate (A) and cover crop treatments (B). Vertical bars indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N; 0 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>).

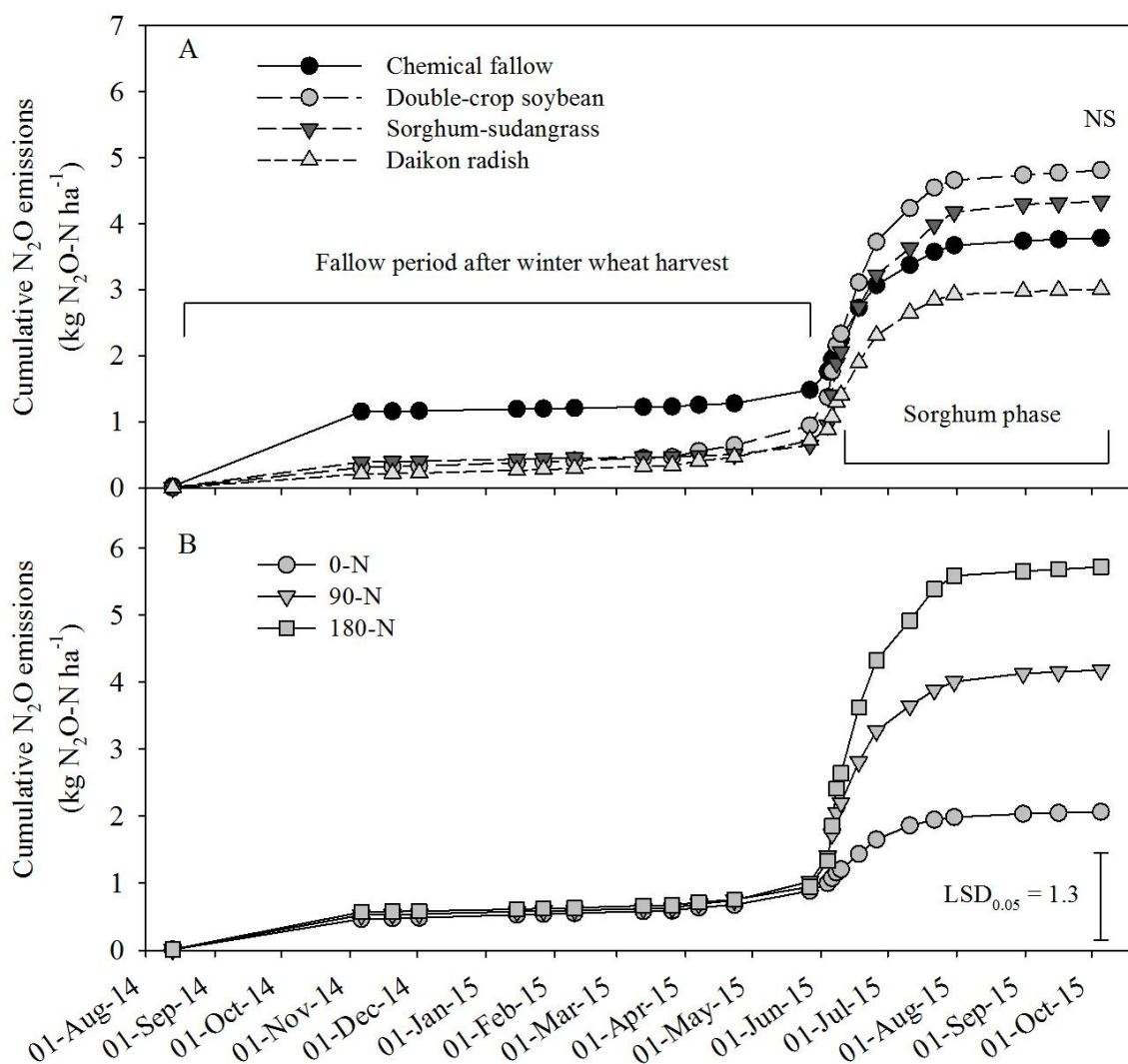


**Figure 3.9.** Grain yield response to cover crops and nitrogen (N) fertilization of sorghum grown in 2015 at Ashland Bottoms, KS. Vertical error bars indicate the 95% confidence interval.

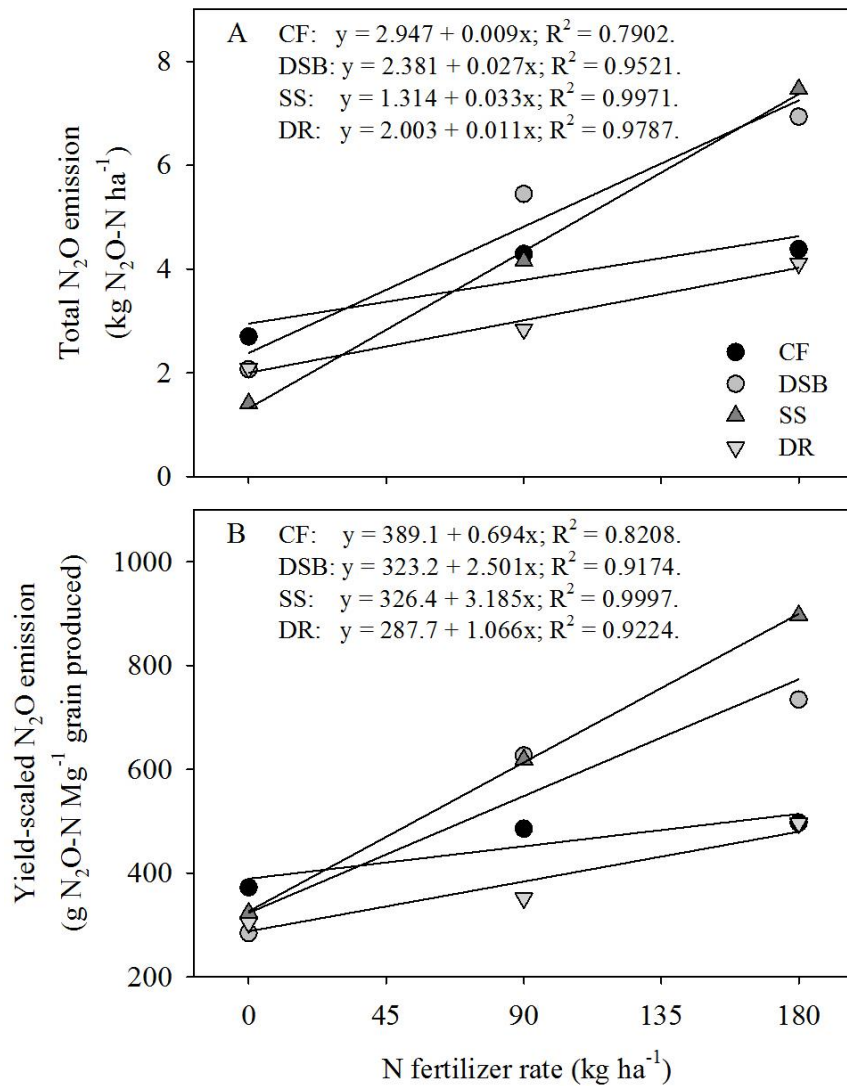




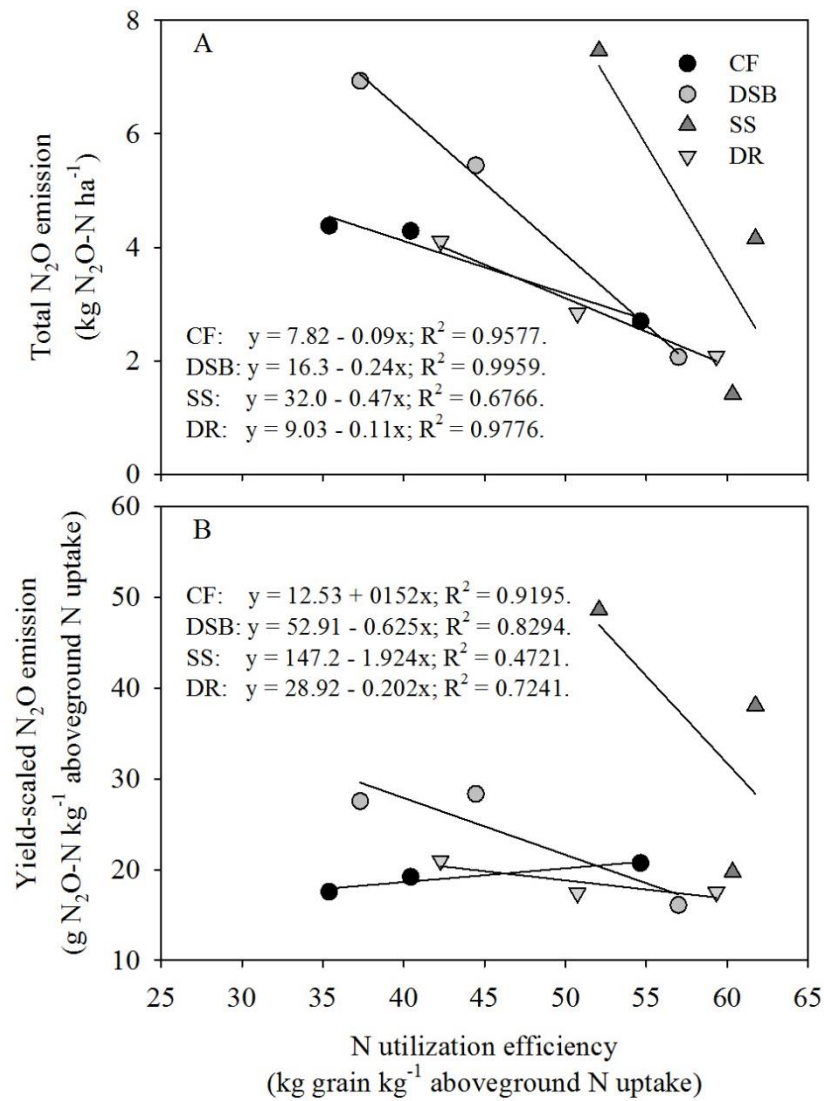
**Figure 3.10.** Nitrogen (N) utilization efficiency of grain sorghum as affected by cover crop and N rate. Vertical bars indicate Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 90-N, kg N ha<sup>-1</sup>; 180-N, kg N ha<sup>-1</sup>; CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).



**Figure 3.11.** Dynamic of cumulative nitrous oxide ( $N_2O$ ) emissions under different cover crop (A) and nitrogen (N) rate treatments (B) from cover crop planting in 2014 through grain sorghum harvesting in 2015 at Ashland Bottoms, KS. Vertical bar at the last sampling event in B indicates the Fisher's LSD at  $P \leq 0.05$ . (0-N; 0  $kg\ N\ ha^{-1}$ ; 90-N, 90  $kg\ N\ ha^{-1}$ ; 180-N, 180  $kg\ N\ ha^{-1}$ ; NS, not significant.).



**Figure 3.12.** Relationship between nitrogen (N) fertilizer rate and total cumulative nitrous oxide (N<sub>2</sub>O) emission (A) and yield-scaled N<sub>2</sub>O emission. Total N<sub>2</sub>O emissions represent values from cover crop planting in 2014 through sorghum harvesting in 2015. (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).



**Figure 3.13.** Relationship between nitrogen (N) utilization efficiency and total cumulative nitrous oxide (N<sub>2</sub>O) emission (A) and yield-scaled N<sub>2</sub>O emission. Total N<sub>2</sub>O emissions represent values from cover crop planting in 2014 through sorghum harvesting in 2015. (CF, chemical fallow; DSB, double-crop soybean; SS, sorghum-sudangrass; DR, daikon radish.).

**Table 3.1.** Average monthly temperature and precipitation at Ashland Bottoms Research Farm in 2014, 2015, and 2016.

Month	Precipitation				Temperature			
	2014	2015	2016	Normal§	2014	2015	2016	Normal§
	----- mm -----				----- °C -----			
Jan	0.5	22.1	12.7	16.0	-2.9	-0.6	-1.7	-1.7
Feb	22.9	10.2	10.6	27.0	-3.3	-2.5	4.3	1.1
Mar	31.1	4.2	11.7	63.0	4.2	8.1	10.3	6.5
Apr	89.1	67.6	214.6	81.0	9.5	13.9	14.5	12.5
May	49.5	218.7	177.3	129.0	19.3	17.6	17.5	18.4
June	176.8	107.2	39.4	145.0	23.8	24.9	26.4	23.7
July	21.1	128.0	154.9	112.0	24.8	26.4	26.8	26.7
Aug	91.4	81.0	185.6	105.0	26.3	23.8	25.0	25.6
Sept	29.0	104.9	105.6	87.0	20.3	23.3	22.2	20.5
Oct	74.4	15.5	70.3	68.0	14.7	15.0	16.8	13.6
Nov	1.3	110.5	7.6	44.0	3.2	9.5	10.5	6.2
Dec	54.1	82.8	16.5	27.0	1.7	3.7	-3.0	-0.5
Total	641.2	952.6	1006.8	904.0	-	-	-	-

§30-yr average, 1981 – 2010 Normal.

**Table 3.2.** ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, date, and their interaction on daily nitrous oxide emissions during the wheat, cover crop, and sorghum phases of the rotation.

<b>Source of variation</b>	<b>Wheat 2014-15</b>	<b>Wheat 2015-16</b>	<b>Cover crop 2014-15</b>	<b>Cover crop 2015-16</b>	<b>Sorghum 2015</b>
Cover crop (CC)	0.013	0.864	< 0.0001	< 0.0001	0.024
N rate (N)	0.117	0.290	0.814	0.627	< 0.0001
CC x N	0.002	0.636	0.038	0.534	0.007
Date (D)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
D x CC	0.473	0.488	< 0.0001	< 0.0001	0.215
D x N	0.592	0.573	0.919	0.953	0.0007
D x CC x N	0.049	0.634	0.261	0.678	0.053

**Table 3.3.** ANOVA significance levels for the effect of cover crops, nitrogen (N) rate, year, and their interactions on total cumulative nitrous oxide emissions at the end of the wheat, cover crop, and sorghum phases of the rotation.

Source of variation	Wheat phase	Cover crop phase	Sorghum phase
Cover crop (CC)	0.516	0.006	0.023
N rate (N)	0.156	0.759	< 0.0001
CC x N	0.095	0.698	0.132
Year (Y)	< 0.0001	0.020	-§
Y x CC	0.0009	0.994	-
Y x N	0.379	0.921	-
Y x CC x N	0.066	0.858	-

§Sorghum data only contain measurements from 2015, therefore there is no effect of year and its interactions.

**Table 3.4.** Effects of cover crop (CC) and nitrogen (N) rate treatments on total aboveground dry matter yield, total N uptake, and grain N yield of sorghum grown in 2015 at Ashland Bottoms, KS.

Treatment	Dry matter yield	Total N uptake	Grain N yield
	Mg ha <sup>-1</sup>	----- kg ha <sup>-1</sup> -----	
		<i>Cover crop</i>	
Chemical fallow	18.88 a§	203.45 a	125.75 a
Double-crop soybean	19.54 a	193.22 a	121.71 a
Sorghum-sudangrass	15.80 b	119.56 c	80.29 c
Daikon radish	18.80 a	158.61 b	104.84 b
		<i>N rate</i>	
0 kg ha <sup>-1</sup>	15.69 c	114.98 c	79.12 c
90 kg ha <sup>-1</sup>	18.90 b	175.52 b	114.51 b
180 kg ha <sup>-1</sup>	20.17 a	215.63 a	130.82 a
		----- Pr > F -----	
CC	<0.0001	<0.0001	<0.0001
N rate	<0.0001	<0.0001	<0.0001
CC x N rate	0.11	0.13	0.68

§ Treatment means within column followed by different letters are significantly different ( $P \leq 0.05$ ).



**Table 3.5.** Effects of cover crop (CC) and nitrogen (N) rate treatments on total cumulative nitrous oxide (N<sub>2</sub>O) emissions at the end of the 2014-15 cover crop and 2015 sorghum phase, and the combined cover crop plus sorghum period.

Treatment	Cover crop phase <sup>1</sup>	Sorghum phase <sup>2</sup>	Cover crop + sorghum <sup>3</sup>
----- kg N <sub>2</sub> O ha <sup>-1</sup> -----			
<i>Cover crop</i>			
Chemical fallow	1.48 a§	2.08 b	3.56 <sup>NS</sup>
Double-crop soybean	0.94 b	3.53 a	4.47
Sorghum-sudangrass	0.65 b	3.45 a	4.10
Daikon radish	0.72 b	2.15 b	2.87
<i>N rate</i>			
0 kg ha <sup>-1</sup>	0.88 <sup>NS</sup>	1.08 c	1.96 c
90 kg ha <sup>-1</sup>	1.02	2.85 b	3.87 b
180 kg ha <sup>-1</sup>	0.94	4.47 a	5.41 a
----- Pr > F -----			
CC	0.01	0.02	0.11
N rate	0.46	<0.0001	<0.0001
CC x N rate	0.51	0.13	0.18

<sup>1</sup>Total cumulative emissions at the end of the cover crop phase.

<sup>2</sup>Total cumulative emissions at the end of the sorghum phase.

<sup>3</sup>Includes total cumulative emissions from cover crop planting in 2014 through sorghum harvest in 2015.

§Treatment means within column followed by different letters are significantly different ( $P \leq 0.05$ ).

NS, not significant.

**Table 3.6.** Effects of cover crop (CC) and nitrogen (N) rate treatments on three different indexes of yield-scaled N<sub>2</sub>O emissions\* (YSNE) and fertilizer induced emission factor\* (FIEF) of sorghum grown in 2015 at Ashland Bottoms, KS.

Treatment	YSNE <sup>1</sup>	YSNE <sup>2</sup>	YSNE <sup>3</sup>	FIEF
	g N <sub>2</sub> O kg <sup>-1</sup> N uptake	g N <sub>2</sub> O kg <sup>-1</sup> grain	g N <sub>2</sub> O kg <sup>-1</sup> N in grain	%
<i>Cover crop</i>				
Chemical fallow	19.1 a§	451 <sup>NS</sup>	30.3 <sup>NS</sup>	1.35 a
Double-crop soybean	23.9 a	469	37.6	3.22 b
Sorghum-sudangrass	35.4 b	613	52.1	3.20 b
Daikon radish	18.6 a	383	28.2	0.98 a
<i>N rate</i>				
0 kg ha <sup>-1</sup>	18.5 a	308 a	26.9 a	-
90 kg ha <sup>-1</sup>	25.7 ab	497 b	38.5 b	2.35 <sup>NS</sup>
180 kg ha <sup>-1</sup>	28.6 b	632 b	45.8 b	2.03
----- Pr > F -----				
CC	0.07	0.22	0.12	0.01
N rate	0.02	0.0005	0.005	0.53
CC x N rate	0.11	0.35	0.16	0.74

\*Includes total cumulative emissions from cover crop planting in 2014 through sorghum harvest in 2015.

<sup>1</sup>N<sub>2</sub>O emission per unit N uptake by aboveground biomass.

<sup>2</sup>N<sub>2</sub>O emission per unit grain produced. Includes grain yield of double-crop soybean plus sorghum.

<sup>3</sup>N<sub>2</sub>O emission per unit N accumulated in grain.

§Treatment means within column followed by different letters are significantly different ( $P \leq 0.05$  or 0.1).

NS, not significant.

## Chapter 4 - General conclusion

Nitrogen (N) is an essential nutrient to crop plants, and it is often the nutrient that most limits yield. The use of N fertilizer significantly increased crop production over the past decades, but it also contributed to environmental problems, such as groundwater pollution, eutrophication, and nitrous oxide (N<sub>2</sub>O) emissions into the atmosphere. Cover crops (CCs) are one strategy to both reduce N loss during the intercrop periods and supply N to the following cash crop. Therefore, identifying the optimum N fertilizer rate applied to major cereal crops, plus the selection of CCs adapted to climatic conditions could be key strategies used to improve the sustainability of no-till cropping systems.

Research objectives were to (i) evaluate the performance of different CCs following winter wheat, (ii) determine the long-term effects (3 cycles over 9 years) of CCs and varying N rates on subsequent sorghum grain yield and yield components, (iii) assess how CCs affect the N dynamic in soil-crop relationship during the growing season and the consequent effect on NUE of sorghum plants, and (iv) define important periods of N<sub>2</sub>O losses and evaluate how different fallow management and N fertilization affects N<sub>2</sub>O emissions throughout the cropping systems.

To answer the research questions, field experiments were conducted during the 2014-15 and 2015-16 growing seasons, within a long-term experiment established in 2007 at Ashland Bottoms Research Farm of Kansas State University, located approximately 8 km south Manhattan, KS. The experiment consisted of all phases of a three-year no-till winter wheat (*Triticum aestivum* L.)/cover crop – sorghum [*Sorghum bicolor* (L.) Moench] – soybean [*Glycine max* (L.) Merr] rotation. Cover crop were grown after wheat harvest, and included late-maturing soybean, sorghum-sudangrass (*Sorghum bicolor* var. Sudanese), crimson clover (*Trifolium incarnatum* L.), daikon radish (*Raphanus sativus* L.), plus a chemical fallow treatment as a control and a double-

crop soybean treatment as a cash crop alternative grown after wheat harvest. The N fertilizer was subsurface banded at five rates (0, 45, 90, 135, and 180 kg ha<sup>-1</sup>) after sorghum planting.

Our results showed that sorghum-sudangrass exhibited the greatest biomass potential, producing, on average, 6.6 Mg ha<sup>-1</sup> during the two study years. If water is not limited, both late-maturing soybean and crimson clover can produce adequate biomass and accumulate significant amounts of N (99 and 80 kg N ha<sup>-1</sup>, respectively) during the traditional summer fallow period, compared to 42 kg N ha<sup>-1</sup> for daikon radish.

In general, the response of sorghum grain yield to N rates was similar for most cover crop treatments. The optimum grain yield occurred at an N rate around 135 kg N ha<sup>-1</sup> for sorghum grown after sorghum-sudangrass and daikon radish, and around 90 kg N ha<sup>-1</sup> for the remaining treatments. The higher yields with lower rates of N applied to sorghum demonstrate the potential of crimson clover, late-maturing soybean, and double-crop soybean to supply N to the following cash crop in the rotation. This conclusion is supported in Chapter 2, by the calculation of the N fertilizer replacement value provided for each cover crop treatment. On average, double-crop soybean and late-maturing soybean provided one-third and one-half of the N required for maximum economic grain yield, respectively.

As has been reported in previous studies, increasing N fertilizer rate decreased the N use efficiency (NUE) of sorghum plants, indicating that the increased soil N supply and/or the increased N uptake by sorghum plants were not associated with a significant increase in grain yield. When averaged across years and N rate, the chemical fallow (control) treatment exhibited lower values of NUE. Considering the optimum N rate of 90 kg ha<sup>-1</sup>, the chemical fallow decreased N utilization efficiency compared to crimson clover, daikon radish, and sorghum-sudangrass. In addition, chemical fallow had N utilization efficiency of 38.4 compared to 41.7 kg grain kg<sup>-1</sup> N

uptake for late-maturing and double-crop soybean, although the difference was not statistically significant. These results show evidences that all CCs and double-crop soybean have a tendency to improve the efficient use of N when N fertilizer rate matches the minimum required for maximum grain yield.

When considering the N dynamics throughout the cropping system, the use of CCs or double-crop soybean after winter wheat were confirmed to be a good strategy to reduce N losses (potential  $\text{NO}_3^-$  leaching and  $\text{N}_2\text{O}$  emissions) during the fallow period, especially during the summer where residual N accumulation and  $\text{N}_2\text{O}$  emissions are expected to be high. The low soil  $\text{NO}_3^-$  concentration, which was related to the N uptake by the CCs and double-crop, explained the lower  $\text{N}_2\text{O}$  fluxes observed throughout the fallow period in comparison to chemical fallow.

However, the use of synthetic N fertilizer in the following grain sorghum crop tended to offset the overall effects of CCs, in which no significant difference was observed among cover crop treatments on the cumulative  $\text{N}_2\text{O}$  emissions at the end of the sorghum phase. Regardless of the cover crop treatment, about 50% of the total emissions occurred within 3 weeks after N application. This result emphasizes the importance of implementing N management strategies to reduce  $\text{N}_2\text{O}$  emissions early in the growing season.

Although sorghum grain yield was influenced by previous cover crop and N rate, the combination of these two treatments did not affect the overall  $\text{N}_2\text{O}$  efficiency of the cropping system, suggesting that the potential increase of  $\text{N}_2\text{O}$  emissions during the fallow period (for chemical fallow) and sorghum phase (double-crop soybean) are being balanced with improved crop yield. Overall, sorghum-sudangrass was the only cover crop that significantly increased yield-scaled  $\text{N}_2\text{O}$  emission. A second year of data from the 2016 sorghum phase will provide a better understanding of the  $\text{N}_2\text{O}$  efficiency of the cropping system.

In theory, CCs and N management (N source, rate, timing, and placement) are key best management practices for reducing soil N losses and synchronizing N availability with periods of high crop demand. However, the results of this study show that CCs selection and N fertilizer management can have significant impacts on sorghum productivity and N<sub>2</sub>O emissions in no-till cropping systems, and a better understanding and evaluation of the tradeoff existing between grain yield and N<sub>2</sub>O emissions are necessary for improving management of N resources in a wheat-sorghum cropping sequence.

## **Appendix A - Assessment of nutrient and pH dynamic after eight years of no-till and cover crops**

### **Abstract**

Long-term soil and nutrient management can increase the productivity and profitability of cropping systems. Therefore, there is a need to assess long-term studies in order to better understand the dynamics of nutrient distribution in the soil profile. This study aimed to evaluate the long-term effects of different fallow and nitrogen (N) fertilizer management options on pH and nutrient distribution in the soil profile in a three-year winter wheat (*Triticum aestivum*) – grain sorghum [*Sorghum bicolor* (L.) Moench] – soybean [*Glycine max* (L.) Merr] rotation. Fallow management consisted of four different cover crops (CCs) and double-crop soybean grown after wheat harvest plus a chemical fallow as a control treatment, and the N fertilizer was subsurface banded at 5 rates (0, 45, 90, 135, and 180 kg ha<sup>-1</sup>) in the sorghum phase of the rotation. Profile soil samples were collected in 2014 and 2015 after sorghum harvest and divided into five depth increments (0-5, 5-10, 10-15, 15-30, and 30-60 cm). Soil samples were analyzed for pH, available phosphorous (P), exchangeable potassium (K), mineral nitrogen (N), and total N and carbon (C). In general, nutrient concentration was higher near soil surface overtime regardless of fallow management; therefore, differences among cover crop treatments were only pronounced at 0-5 cm depth for pH, P, total N and C. Exchangeable K followed a distinct distribution in both years, with high concentration near soil surface and deeper in the profile. Overall, these results suggest that the inclusion of CCs into the rotation is not significantly enhancing nutrient stratification after eight years of no-till.

Abbreviations: 0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; CCs, cover crops; P, phosphorous; K, potassium; NO<sub>3</sub><sup>-</sup>, nitrate; NH<sub>4</sub><sup>+</sup>, ammonium, SOC, soil organic carbon.

## **Material and methods**

### ***Site description and Experimental Design***

This study was based on a long-term experiment established in 2007 at Ashland Bottoms Research Farm of Kansas State University (K-State), located approximately 8 km south of Manhattan, KS (39° 11' N lat, 96° 35' W long). The altitude is 311 m. The region has a humid subtropical climate (Köppen Climate Classification System: Cfa), with an average annual precipitation of 904 mm. The annual mean temperature is 12.7 °C. The soil was a moderately-well drained Wymore silty clay loam (fine, smectitic, mesic Aquertic Argiudoll), with 0 to 1% slopes.

The experiment consisted of a no-till three-year winter wheat/ cover crop – grain sorghum – soybean rotation, where each component of the rotation was present every year. The experiment was arranged in a split-plot randomized complete block design with four replications. The cover crop treatments were the whole plots and N rates were the subplots. Each whole plot was 6 by 70 m and each subplot was 6 by 14 m. The cover crop treatments included a summer legume, summer non-legume, winter legume, winter non-legume cover crop, plus a chemical fallow plot as a control and a double-crop soybean plot as a cash crop alternative following winter wheat harvest. The N fertilizer treatments consisted of a control (zero N) and four N rates (45, 90, 135, and 180 kg ha<sup>-1</sup>; hereafter, 0-N, 45-N, 90-N, 135-N, and 180-N, respectively) that were subsurface banded as 28% urea ammonium nitrate (UAN) after sorghum planting.

### ***Winter wheat and cover crop phase***



Winter wheat was planted immediately after soybean harvest in late-October to early-November in all years, with a target seeding rate of 115 kg ha<sup>-1</sup> in 19-cm rows using a John Deere 1590 no-till drill (Deere & Co., Moline, IL). Diammonium phosphate was applied at planting with wheat seed at a rate of 65 kg ha<sup>-1</sup>. On March of the following year, the wheat was top-dressed with 67 kg N ha<sup>-1</sup> soon after spring green-up using UAN, applied in streams spaced every 10 cm. The weeds were controlled by applying glyphosate [N-(phosphonomethyl)glycine] at a rate of 1.67 kg a.i. ha<sup>-1</sup> the next day after planting, and a herbicide in the form of 21 g a.i. ha<sup>-1</sup> of thifensulfuron-methyl plus 10.5 g a.i. ha<sup>-1</sup> of tribenuron-methyl (Harmony® Extra SG, DuPont) on March. Winter wheat was harvested late-June to early-July.

The cover crop management varied during the entire experiment. The summer cover crops (CCs) and double-crop soybean were planted immediately after wheat harvest. Cover crop species included sorghum-sudangrass (*Sorghum vulgare* var. sudanese) as summer non-legume and late maturing soybean [*Glycine max* (L.) Merr] as summer legume in all years. The target seeding rate was 23 kg ha<sup>-1</sup> for sorghum-sudangrass and 420,000 seeds ha<sup>-1</sup> for late-maturing soybean and double-crop soybean. After CCs and double-crop soybean planting, the plots were sprayed with glyphosate at a rate of 1.67 kg a.i. ha<sup>-1</sup>. Summer CCs were terminated using a rotary mower at beginning of flowering (10-12 weeks after planting) during the first 2 years of the experiment, thereafter, a roller-crimper was used to terminate the summer CCs. Double-crop soybean plots were harvested with a plot combine on late-October to early-November in all years.

Between 2007 and 2011, canola (*Brassica napus* L.) was used as winter non-legume cover crop. Winter pea (*Pisum sativum*) was used as winter legume cover crop between 2007 and 2010, whereas red clover (*Trifolium pretense* L.) was grown in 2010 and 2011. In all years, canola was planted at 11 kg ha<sup>-1</sup> and winter pea was planted at 30 kg ha<sup>-1</sup>. Beginning in 2012, tillage radish

(*Raphanus sativus* L.) and crimson clover (*Trifolium incarnatum* L.) were used as winter non-legume and winter legume cover crop, respectively. The target seeding rate was 12 kg ha<sup>-1</sup> for tillage radish and 23 kg ha<sup>-1</sup> for crimson clover in all years. The winter CCs were planted in late-August to early-September until 2013, and planted together with summer CCs thereafter. On March of the following year after CCs planting, all plots were sprayed with 1.46 kg a.i. ha<sup>-1</sup> of glyphosate plus 0.53 kg a.i. ha<sup>-1</sup> of 2,4-D (2,4-Dichloro-phenoxyacetic acid) to kill volunteer wheat, CCs, and other winter annual weeds.

### ***Grain sorghum phase***

Grain sorghum was planted mid-May to early-June in all years, with a target seeding rate of 119,000 seeds ha<sup>-1</sup>. Planting was performed using a White 6200 4-row planter (AGCO Corp., Duluth, GA) with a 76-cm row spacing. Planting depth was 2.5 to 3.5 cm. Nitrogen fertilizer rates of 45, 90, 135, and 180 kg ha<sup>-1</sup> were subsurface banded after sorghum planting using urea ammonium nitrate (28% UAN) with a straight flat-coulter liquid fertilizer applicator to inject N fertilizer below the residue layer. No phosphorus or potassium fertilizer was applied in all years. Weeds were controlled by applying 1.25 kg a.i. ha<sup>-1</sup> of glyphosate and a herbicide in the form of 2.26 kg a.i. ha<sup>-1</sup> of acetochlor [2-chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl)acetamide] and 1.12 kg a.i. ha<sup>-1</sup> of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] (Degree Xtra®, Monsanto) within 2 days after planting in all years. Sorghum plots were harvested in October of all years.

### ***Soybean phase***

In all years, planting dates for soybean varied from mid-May to mid-June. Maturity group between 3.4 and 3.6 were planted at a seeding rate of 432,000 seeds ha<sup>-1</sup>, with 76-cm row spacing. Ammonium polyphosphate was applied as a starter fertilizer with planter at a rate of 130 kg ha<sup>-1</sup>.

No potassium fertilizer was applied in all years. Before soybean planting (in March), all plots were sprayed with 1.46 kg a.i. ha<sup>-1</sup> of glyphosate plus 0.53 kg a.i. ha<sup>-1</sup> of 2,4-D to kill winter annual weeds. After planting, weeds were controlled by applying 1.46 kg a.i. ha<sup>-1</sup> of glyphosate plus 1.56 kg a.i. ha<sup>-1</sup> of alachlor, 2-chloro-2,6-diethyl-N-(methoxymethyl)acetanilide (Intrro®, Monsanto) as a pre-emergence herbicide. Soybean was harvested in October of all years.

### ***Soil sampling and laboratory analysis***

Four soil cores (4-cm diameter) were taken from each treatment plot after sorghum harvest on November 2014 and 2015. The cores were collected to a 60 cm depth using a tractor mounted hydraulic probe, divided into five depth increments (0-5, 5-10, 10-15, 15-30, and 30-60 cm), and composited for a total of five samples per plot. Composite samples were dried in a forced-air oven at 40 °C until constant weight, ground to pass a 2-mm sieve using a soil grinder equipped with a ceramic mortar, ceramic screw type grinding head, and stainless steel screen (Nasco-Asplin, Fort Atkinson, WI), and submitted for analysis following procedures of the K-State Soil Testing Laboratory. Briefly, soil pH was determined using a 1:1(v:v) soil:water mixture. Mehlich-3 method was used to determine P concentrations (Frank et al., 1998). Available K levels were determined by the ammonium acetate (1 M NH<sub>4</sub>OAc at pH 7.0) extraction method (Warncke and Brown, 1998). Total C and N was analyzed by direct combustion using a C/N analyzer (Model LECO TruSpec, LECO Corporation, St. Joseph, MI). Total C was assumed to be equivalent to soil organic C (SOC) due to low pH and no presence of carbonate in the soil. For soil-extractable nitrate-N (NO<sub>3</sub><sup>-</sup>-N), a 1 M potassium chloride extraction (2g of soil in 20 mL extraction, shaken for 15 min) and cadmium reduction/colorimetry were used, according to Gelderman and Beegle (1998). Ammonium-N (NH<sub>4</sub><sup>+</sup>-N) was extracted from soil samples with 1 M potassium chloride

(2g of soil in 20 mL extraction, shaken for 30 min) and measured by an indophenol colorimetric reaction as described by Keeney and Nelson (1982).

### ***Statistical analysis***

Data were analyzed using a split-plot randomized complete block design, with cover crop as the whole plot factor and N rate as the subplot factor. The effects of cover crop, N rates, and their interaction on nutrient concentration at each depth were evaluated by ANOVA. Year was included in the analysis and treated as a fixed factor to determine interactions involving year. Fisher's Least Significant Difference (LSD) method was used to assess the difference between the means of the treatments. The ANOVA and mean separation differences were carried out using PROC MIXED of the SAS® software (SAS Institute Inc, Cary, NC). All results were considered significantly different at  $P \leq 0.05$ .

## **Results**

### ***pH***

The interaction between cover crop and year significantly affected pH only at the surface 0-5 cm depth (Table A.1). The effects of CCs on the pH distribution in the soil profile followed similar patterns in both years, that is, increased values with increasing depth. In 2014, pH was greater after daikon radish when compared to late-maturing soybean, crimson clover, and chemical fallow, but it was not statistically different from double-crop soybean and sorghum-sudangrass (Fig A.1a). In 2015, daikon radish and sorghum-sudangrass had the greatest pH when compared to the other CCs and chemical fallow treatments (Fig A.1b).

N rate also affected pH only at the 0-5 cm depth. When averaged across years and cover crop treatments, pH was significantly greater when no N was applied (Fig A.2). The application

of 180-N significantly reduced pH compared to 90-N and 45-N, but it was not different from 135-N.

### ***Mehlich-3 phosphorous***

The cover crop X year interaction was significant only at the 0-5 cm depth (Table A.1). In general, concentration of P was higher near the soil surface and abruptly declined with increasing depth in both years (Fig A.3). When averaged across N rate, double-crop soybean reduced soil available P in both years (25.25 and 18.8 mg kg<sup>-1</sup> in 2014 and 2015, respectively). In 2014, no difference was observed among the CCs and chemical fallow treatments, with concentrations ranging from 30.9 to 39.4 mg kg<sup>-1</sup> (Fig A.3a). In 2015, daikon radish significantly increased P concentration (37.7 mg kg<sup>-1</sup>), whereas no difference was observed among other CCs and chemical fallow treatments, with concentrations varying between 22.3 and 27.8 mg kg<sup>-1</sup> (Fig A.3b).

### ***Exchangeable potassium***

The interaction between cover crop and year was significant deeper in the profile (15-30 cm) (Table A.1). At other soil depths, the main effects and their interaction were not significant (except year). In general, concentration of K followed a distinct distribution in both years, with high concentration near soil surface (0-5 cm), low from 5 to 15 cm, and an increase deeper in the profile (Fig A.4). However, differences among cover crop treatments were only pronounced between years.

### ***Ammonium***

Cover crop X year interaction was significant at 5-10, 10-15, and 15-30 cm depth (Table A.1). At other soil depths, the main effects and their interaction were not significant (except year). In general, higher concentration of NH<sub>4</sub><sup>+</sup> was observed in 2014 than in 2015 (Fig A.5). In addition, differences among CCs varied by depth (Fig A.5a). At the 5-10 cm depth, late-maturing soybean

and sorghum-sudangrass had higher concentrations when compared to the other CCs and chemical fallow. Those effects persisted at deeper depths, but differences were only significant from chemical fallow and crimson clover at 10-15 cm, and from double-crop soybean at 15-30 cm depth. No difference among cover crop treatments was observed in 2015 (Fig A.5b).

### ***Nitrate***

The interaction of cover crop and N rate was significant at 5-10, 10-15, and 15-30 cm depth, and therefore, data was pooled by year (Table A.1). When averaged across years, differences among cover crop treatments were only pronounced when 135-N and 180-N was applied in the cropping system, and they varied by depth (Fig A.6). At 135-N, double-crop and late-maturing soybean increased  $\text{NO}_3^-$  concentration at 5-10 cm when compared to the other CCs and chemical fallow; whereas at 10-15 and 15-30 cm depth, the increased concentration of  $\text{NO}_3^-$  only persisted in plots with double-crop soybean (Fig A.6d). Similar results were found at 180-N, where double-crop soybean significantly increased  $\text{NO}_3^-$  concentration deeper in the profile (Fig A.6e). In addition, sorghum-sudangrass reduced  $\text{NO}_3^-$  content at the three depths.

### ***Total N***

The interactions between N rate and year, and cover crop  $\times$  year were significant at 0-5 and 5-15 cm depth (Table A.1). At other soil depths, the main effects and their interaction were not significant (except cover crop and year). Overall, the distribution of total N in the soil profile was different between years, regardless of CCs and N rate (Fig A.7). In 2015, concentration of total N was higher near the surface and decreased with increasing depth, whereas in 2014, a slight increase occurred at the 15-30 cm depth.

When averaged across N rate, differences among cover crop treatments were only seen in 2015 (Fig A.7a and b). In that year, chemical fallow had the lowest total N content when compared

to other CCs and double-crop soybean, but it was not statistically different from crimson clover (Fig A.7b). The effects of N rate varied between years when averaged across cover crop treatments (Fig A.7c and d). In 2014, 45-N had the highest concentration of total N, whereas it had the lowest in 2015. In 2015, total N concentration was higher at 90-N when compared to other N rates, but it was not different from 180-N (Fig A.7d).

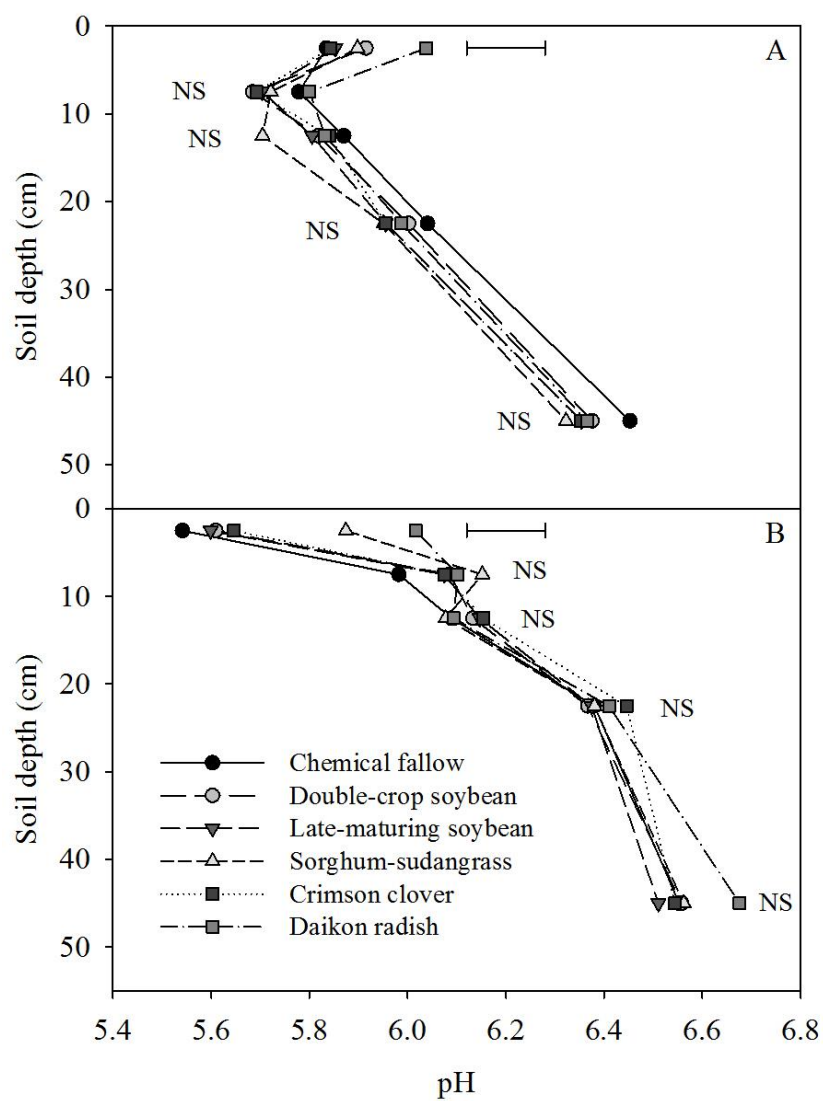
### ***Soil organic C***

Cover crop and N rate  $\times$  year were significant only near surface (0-5 cm) (Table A.1). When averaged across years and N rates, chemical fallow significantly reduced SOC content when compared to the CCs and double-crop soybean, except late-maturing soybean (Fig A.8).

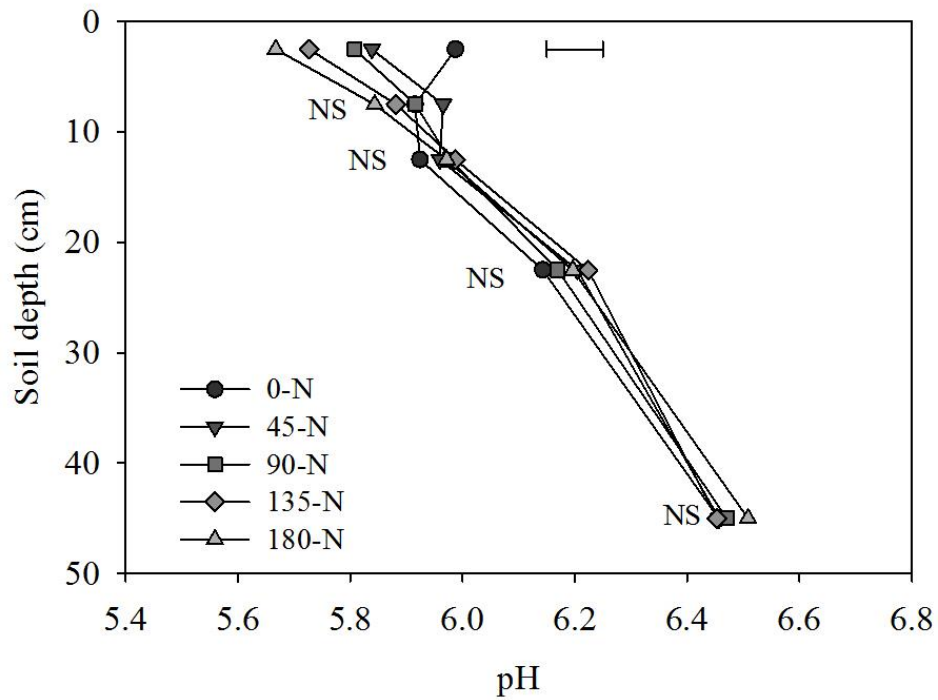
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- Keeney, D.R. and D.W. Nelson. 1982. Nitrogen - inorganic forms. p. 643-698. In A.L. Page (ed.) Methods of soil analysis: Part 2. American Society of Agronomy, Soil Science Society of America, Madison, WI.
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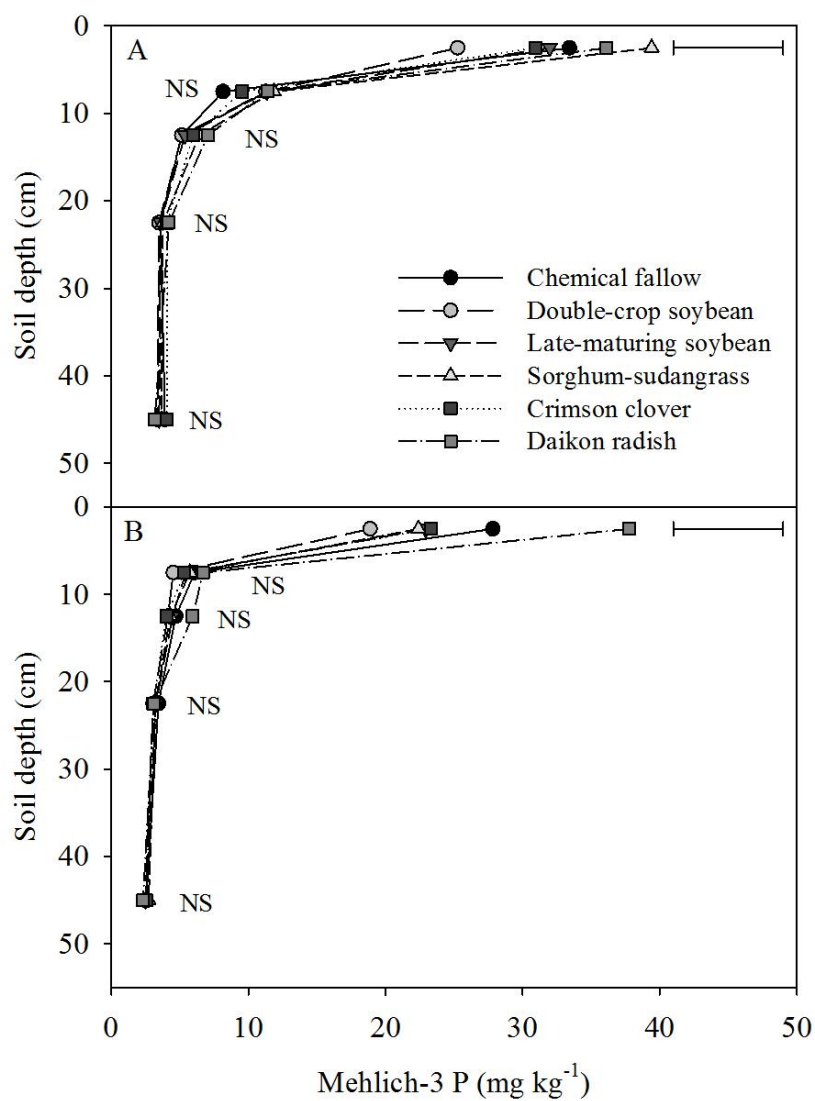




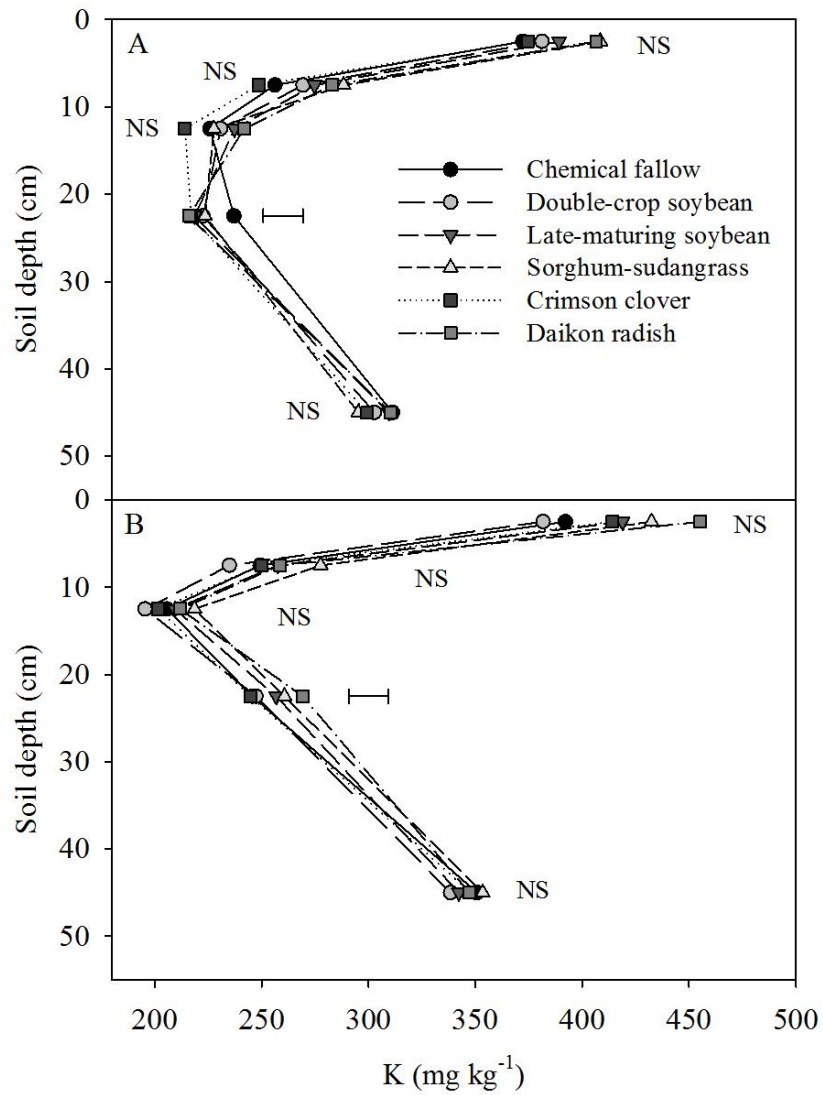
**Appendix A. 1.** Effects of fallow management on pH distribution with depth in 2014 (A) and 2015 (B). Horizontal bars indicate the Fisher's LSD at  $P \leq 0.05$ . (NS, not significant.)



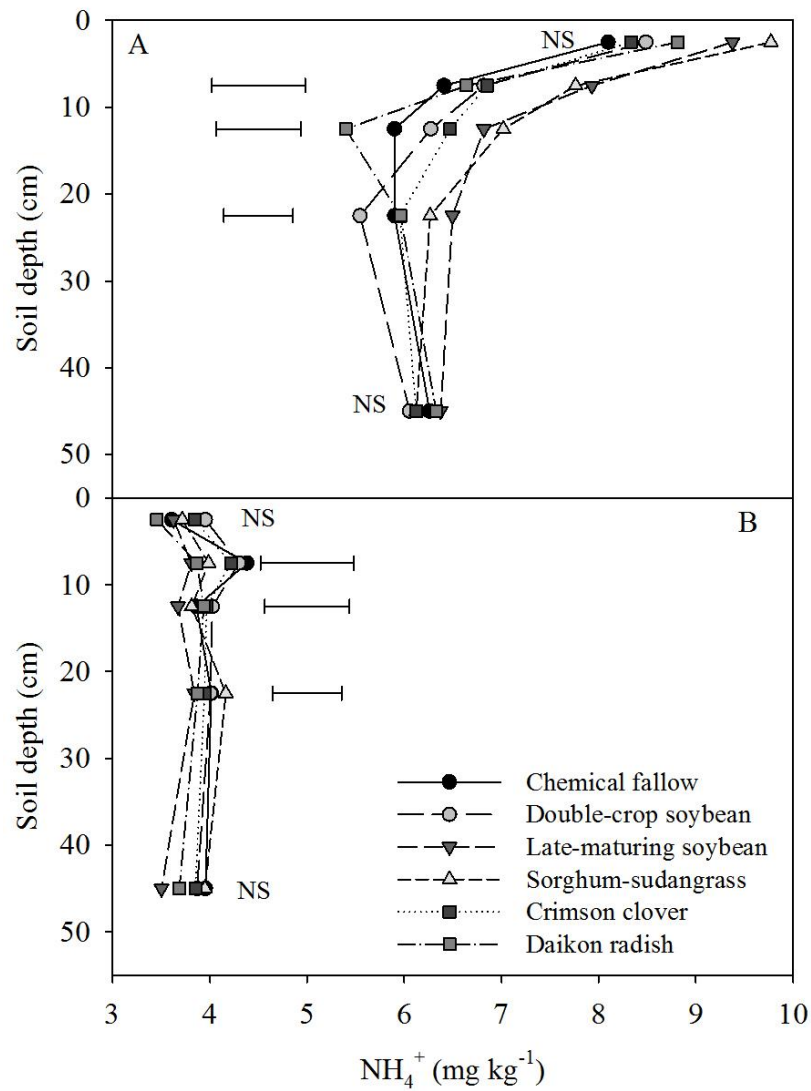
**Appendix A. 2.** Vertical distribution of pH in the soil profile as affected by nitrogen (N) management. Horizontal bar indicates the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; NS, not significant.).



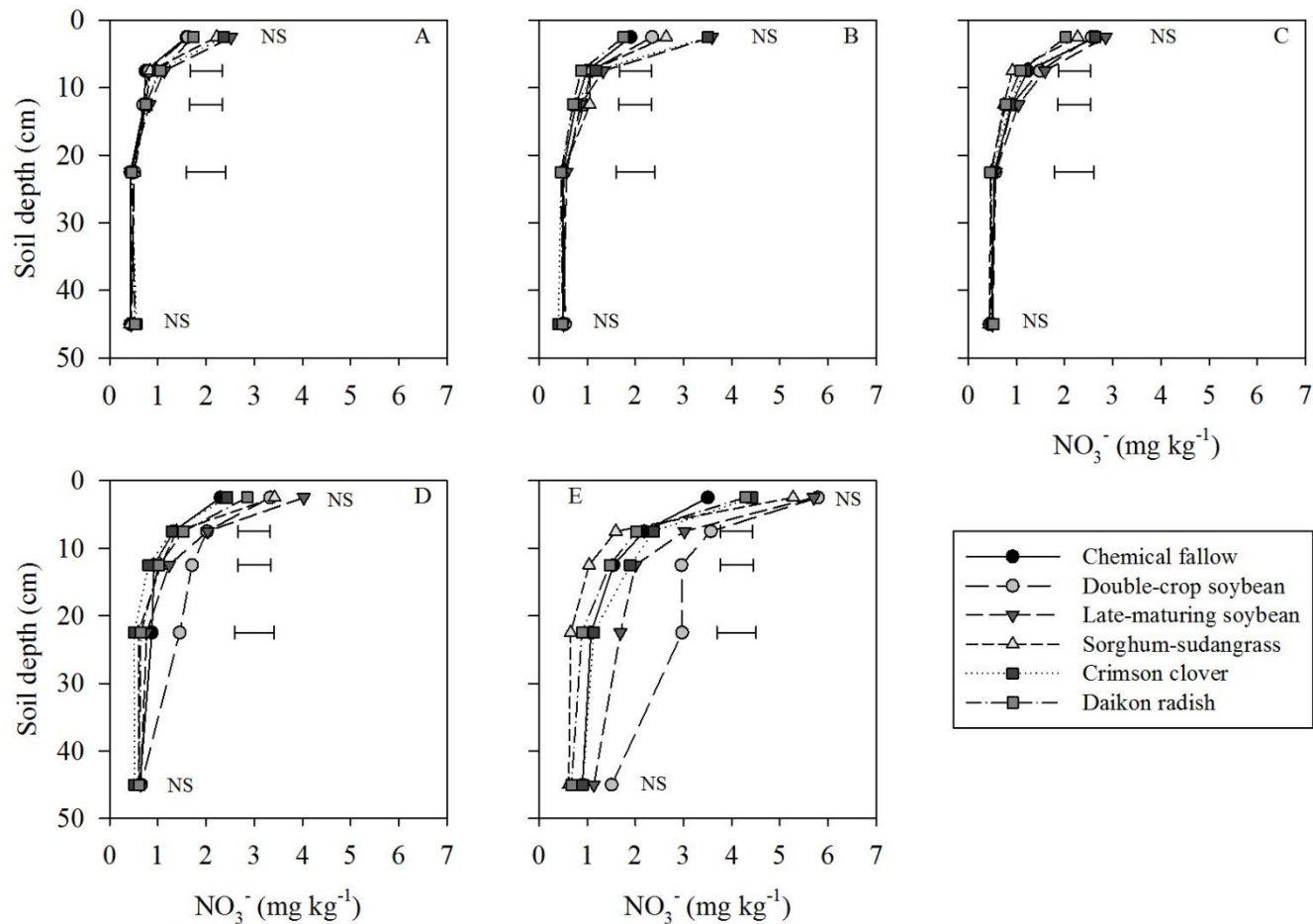
**Appendix A. 3.** Effects of fallow management on mehlich-3 phosphorus (P) concentration in the soil profile in 2014 (A) and 2015 (B). Horizontal bars indicate the Fisher's LSD at  $P \leq 0.05$ . (NS, not significant.).



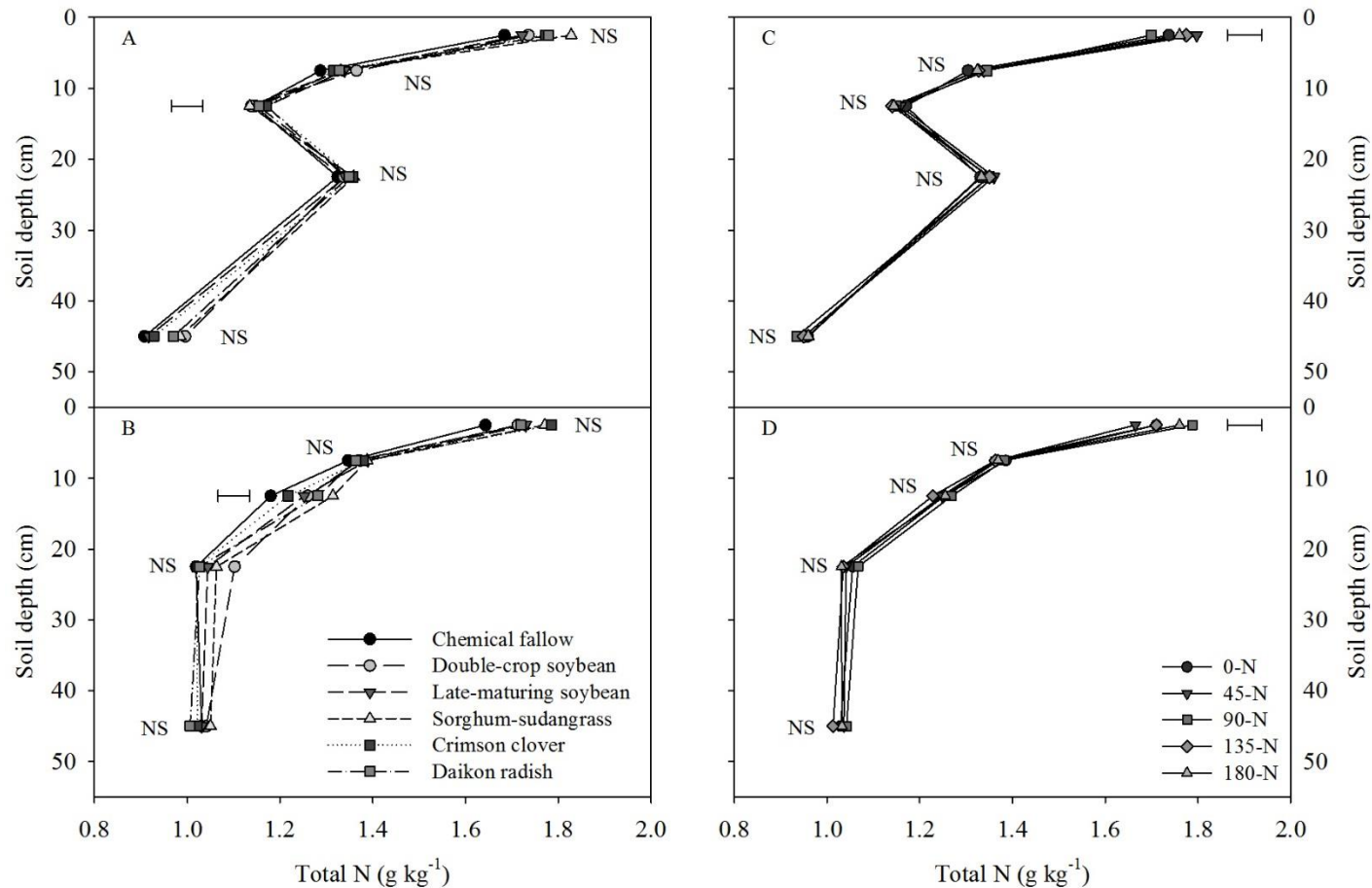
**Appendix A. 4.** Effects of fallow management on exchangeable potassium (K) concentration in the soil profile in 2014 (A) and 2015 (B). Horizontal bars indicate the Fisher's LSD at  $P \leq 0.05$ . (NS, not significant.).



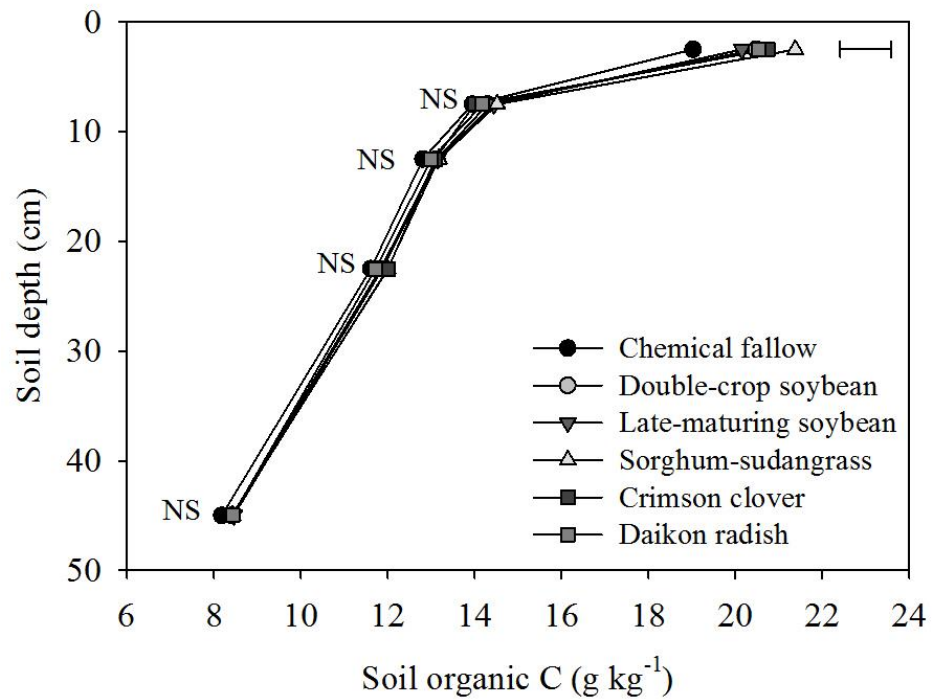
**Appendix A 5.** Vertical distribution of ammonium-N ( $\text{NH}_4^+$ ) in the soil profile as affected by cover crops in 2014 (A) and 2015 (B). Horizontal bars at each depth indicate the Fisher's LSD at  $P \leq 0.05$ . (NS, not significant.).



**Appendix A. 6.** Mean nitrate-N ( $\text{NO}_3^-$ ) concentration in the soil profile as affected by fallow and nitrogen (N) management. Horizontal bars at each depth indicate the Fisher's LSD at  $P \leq 0.05$ . (A, 0 kg N  $\text{ha}^{-1}$ ; B, 45 kg N  $\text{ha}^{-1}$ ; C, 90 kg N  $\text{ha}^{-1}$ ; D, 135 kg N  $\text{ha}^{-1}$ ; E, 180 kg N  $\text{ha}^{-1}$ ; NS, not significant.).



**Appendix A. 7.** Total nitrogen (N) concentration with soil depth as affected by fallow (A and B) and nitrogen (N) management (C and D) in 2014 and 2015, respectively. Horizontal bars at each depth indicate the Fisher's LSD at  $P \leq 0.05$ . (0-N, 0 kg N ha<sup>-1</sup>; 45-N, 45 kg N ha<sup>-1</sup>; 90-N, 90 kg N ha<sup>-1</sup>; 135-N, 135 kg N ha<sup>-1</sup>; 180-N, 180 kg N ha<sup>-1</sup>; NS, not significant.).



**Appendix A. 8.** Vertical distribution of soil organic carbon (C) in the soil profile as affected by cover crops. Horizontal bars at each depth indicate the Fisher's LSD at  $P \leq 0.05$ . (NS, not significant.).



**Table A. 1.** Summary of the ANOVA significance levels within soil depth for pH, Mehlich-3 phosphorus (P), exchangeable potassium (K), nitrate-N ( $\text{NO}_3^-$ ), ammonium-N ( $\text{NH}_4^+$ ), total nitrogen (N), and total organic carbon (C).

Source of variation	Depth				
	0 - 5 cm	5 - 10 cm	10 - 15 cm	15 - 30 cm	30 - 60 cm
----- pH -----					
Cover crop (CC)	0.0009	0.544	0.357	0.895	0.772
Nitrogen (N)	< 0.0001	0.078	0.721	0.382	0.785
Year (Y)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CC x N	0.303	0.664	0.943	0.994	0.788
CC x Y	0.026	0.186	0.728	0.678	0.542
N x Y	0.280	0.465	0.753	0.672	0.508
CC x N x Y	0.084	0.230	0.861	0.906	0.841
----- Mehlich-3 P -----					
CC	0.021	0.838	0.033	0.849	0.424
N	0.210	0.152	0.179	0.694	0.387
Y	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CC x N	0.062	0.397	0.344	0.631	0.964
CC x Y	0.022	0.408	0.195	0.110	0.799
N x Y	0.122	0.492	0.731	0.623	0.362
CC x N x Y	0.688	0.529	0.870	0.950	0.954
----- Exchangeable K -----					
CC	0.177	0.158	0.336	0.547	0.612
N	0.721	0.403	0.959	0.822	0.270
Y	0.0007	0.001	< 0.0001	< 0.0001	< 0.0001
CC x N	0.499	0.988	0.973	0.8872	0.748
CC x Y	0.600	0.326	0.116	0.008	0.293
N x Y	0.190	0.284	0.594	0.179	0.932
CC x N x Y	0.796	0.737	0.957	0.972	0.780
----- $\text{NO}_3^-$ -----					
CC	0.092	0.005	0.086	0.160	0.386
N	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Y	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CC x N	0.911	0.027	0.029	0.006	0.346
CC x Y	0.063	0.0006	0.148	0.380	0.390
N x Y	0.217	< 0.0001	< 0.0001	0.136	0.940
CC x N x Y	0.345	0.020	0.141	0.843	0.521
----- $\text{NH}_4^+$ -----					
CC	0.628	0.526	0.385	0.704	0.997

N	0.188	0.594	0.676	0.913	0.581
Y	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CC x N	0.989	0.782	0.135	0.808	0.925
CC x Y	0.343	0.001	0.003	0.039	0.300
N x Y	0.973	0.600	0.453	0.912	0.375
CC x N x Y	0.999	0.860	0.248	0.384	0.416
----- Total N -----					
CC	0.018	0.667	0.306	0.083	0.115
N	0.703	0.889	0.638	0.566	0.958
Y	0.116	0.0006	< 0.0001	< 0.0001	< 0.0001
CC x N	0.762	0.984	0.907	0.140	0.604
CC x Y	0.723	0.798	0.003	0.102	0.248
N x Y	0.001	0.682	0.810	0.591	0.818
CC x N x Y	0.743	0.680	0.639	0.357	0.813
----- Soil organic C -----					
CC	0.015	0.268	0.360	0.374	0.959
N	0.760	0.580	0.296	0.782	0.890
Y	0.0005	< 0.0001	0.016	0.176	0.575
CC x N	0.488	0.952	0.600	0.959	0.827
CC x Y	0.486	0.545	0.389	0.118	0.287
N x Y	0.031	0.534	0.887	0.850	0.798
CC x N x Y	0.991	0.913	0.845	0.988	0.997

## Appendix B - SAS code

### Chapter 2 – Two-way ANOVA for cover crop biomass, N uptake, N content and C:N ratio

```
data cc;
  input year block ccrop$ nitrogen plot DM_kgha Nuptake_kgha Nperc Cperc C_N;
  cards;
  . . .
  ;

proc sort data=cc;
  by year nitrogen ccrop;
run;
proc print data=cc; run;

proc means data=cc;
  by year nitrogen; class ccrop; var DM_kgha Nuptake_kgha C_N;
run;

%macro mixanova/parmbuff;

  %PUT ***Syspbuff contains: &syspbuff***;
  %let num=1;
  %let respvar=%scan(&syspbuff,&num);
  %do %while(&respvar ne);

proc glimmix=cc plots=studentpanel;
  class year block ccrop nitrogen; by year;
  model &respvar = ccrop|nitrogen / ddfm=kr;
  random block block(ccrop);
  lsmeans ccrop|nitrogen / lines cl pdiff;

  %let num=%eval(&num+1);
  %let respvar=%scan(&syspbuff,&num);
  %end;
%mend mixanova;

%mixanova (DM_kgha Nuptake_kgha DM_lbac Nperc Cperc C_N);

RUN; QUIT;
```

## Chapter 2 – Three-way ANOVA for NDVI, grain yield and yield parameters, and N use efficiencies

```

data sorghum;
input year block plot nitrogen ccrop$ NDVI1 NDV2 YLD_Mgha DM_Mgha
Nuptake_kgha NUE NUtE NUpE Nrecovery NHI FUE;
cards;
. . .
;

proc sort data=sorghum;
  by year nitrogen ccrop;
run;

proc print data=sorghum; run;

proc means data=sorghum;
  by year nitrogen; class ccrop;
  var YLD_Mgha DM_Mgha Nuptake_kgha NUE NUtE NUpE Nrecovery NHI FUE;
run;

%macro mixanova/parmbuff;

  %PUT ***Syspbuff contains: &syspbuff***;
  %let num=1;
  %let respvar=%scan(&syspbuff,&num);
  %do %while(&respvar ne);

proc glimmix=sorghum plots=studentpanel;
  class year block ccrop nitrogen;
  model &respvar = ccrop|nitrogen|year / ddfm=kr;
  random block block(ccrop);
  lsmeans ccrop|nitrogen|year / lines cl pdiff;

  %let num=%eval(&num+1);
  %let respvar=%scan(&syspbuff,&num);
  %end;
%mend mixanova;

%mixanova (YLD_Mgha DM_Mgha Nuptake_kgha NUE NUtE NUpE Nrecovery NHI FUE);

RUN; QUIT;

*Linear and quadratic contrast of N fertilizer on grain yield;
proc glimmix data=sorghum plots=studentpanel;
  class year block ccrop nitrogen; by ccrop;
  model YLD_Mgha = nitrogen|year / ddfm=kr;
  random block block(ccrop);
  contrast 'linear' nitrogen -2 -1 0 1 2;
  contrast 'quadratic' nitrogen 2 -1 -2 -1 2;
run;

```

```

*Nitrogen replacement value by each cover crop;
data sorghum14;
    input nitrogen yld;
    yld2 = yld*yld;
    log_yld = log(yld);
    inv_yld = 1/yld;
    n2 = nitrogen*nitrogen;
cards;
. . .
;

data sorghum15;
    input nitrogen yld;
    yld2 = yld*yld;
    log_yld = log(yld);
    inv_yld = 1/yld;
    n2 = nitrogen*nitrogen;
cards;
. . .
;

*Regression equations;
proc reg data=sorghum14 plots=diagnostics;
    linear: model nitrogen = yld/ p clm clb cli;
    quadratic1: model nitrogen = yld2 / p clm clb cli;
    quadratic2: model nitrogen = yld yld2 / p clm clb cli;
    log_x: model nitrogen = log_yld/ p clm clb cli; *log y;
    inv_yld: model nitrogen = inv_yld / p clm cli clb; *log 1/y;
run;

proc reg data=sorghum15 plots=diagnostics;
    linear: model nitrogen = yld/ p clm clb cli;
    quadratic1: model nitrogen = yld2 / p clm clb cli;
    quadratic2: model nitrogen = yld yld2 / p clm clb cli;
    log_x: model nitrogen = log_yld/ p clm clb cli; *log y;
    inv_yld: model nitrogen = inv_yld / p clm cli clb; *log 1/y;
run;

```

## Chapter 3 – Daily, cumulative, and yield-scaled nitrous oxide emissions

```
data wheat14;
  input date : MMDDYY8. dates block plot ccrop$ nitrogen gas$ adjf cflux;
  format date MMDDYY8.;
  cards;
  . . .
;

proc print data=wheat (obs=20); run;

title '2014-15 Wheat phase - Daily flux - lognormal distribution';
proc glimmix data=wheat14 plots=studentpanel;
  class date block ccrop nitrogen;
  model adjf = ccrop|nitrogen|date / dist=lognormal;
  random _residual_ / type=ante(1) subject=block(ccrop*nitrogen);
  lsmeans date*ccrop*nitrogen / slicediff=nitrogen cl;
run;

*cumulative flux;
Data both_years;
  input year block plot ccrop$ nitrogen cflux;
  cards;
  . . .
;

title '3-way ANOVA for cumulative flux';
proc glimmix data=both plots=studentpanel;
  class year block ccrop nitrogen;
  model cflux = ccrop|nitrogen|year / ddfm=kr;
  random block block*ccrop;
  lsmeans ccrop|nitrogen|year / lines pdiff;
  ods output diffs=diffs;
run;

title 'Calculating Fishers LSD values';
data lsd;
  set diffs;
  lsd = stderr*tinv(0.975,df); *(1-alpha/2,df);
run;

proc print data=lsd; run;

*Same code was used for the Cover crop and Sorghum phase analysis.
```

```

/*****
ANOVA for YSNE
YSNE values represents the total cumulative emissions from 2014 cover crop
planting to 2015 sorghum harvest
YSNE1 = CFlux/total N in grain -> g N2O emitted per kg N in grain
YSNE2 = CFlux/Total N uptake in aboveground biomass -> g N2O emitted per kg N
uptake by aboveground biomass
YSNE3 = CFlux/grain yield -> g N2O emitted per Mg of grain produced
YSNE4 = CFlux/grain yield DSB + Sorghum -> g N2O emitted per Mg of grain
produced in the system
*****/

data ysne;
  input block plot ccrop$ nitrogen CFlux_kgha CFlux_gha Ngrain_kgha
  Nuptake_kgha YLD_Mgha YSNE1 YSNE2 YSNE3 YSNE4;
  Nsurplus = Nuptake_kgha - nitrogen;
  cards;
  . . .
  ;

%macro mixanova/parmbuff;

  %PUT ***Syspbuff contains: &syspbuff***;
  %let num=1;
  %let respvar=%scan(&syspbuff,&num);
  %do %while(&respvar ne);

proc glimmix data=ysne plots=studentpanel;
  class block ccrop nitrogen;
  model &respvar = ccrop|nitrogen / ddfm=kr;
  random block block(ccrop);
  lsmeans ccrop|nitrogen / lines cl pdiff;

%let num=%eval(&num+1);
  %let respvar=%scan(&syspbuff,&num);
  %end;
%mend mixanova;

%mixanova(CFlux_kgha Ngrain_kgha Nuptake_kgha YLD_Mgha YSNE1 YSNE2 YSNE3
YSNE4);

RUN; QUIT;

```

## Appendix A – Three-way ANOVA for nutrient and pH by depth

```
data strat;
  input year depth block plot ccrop$ nitrogen pH P K NH4 NO3 TotalN_perc
TotalC_perc OM_perc;
  cards;
  . . .
;

proc sort data=strat;
  by depth year ccrop nitrogen;
run;

proc print data=strat; run;

%macro mixanova/parmbuff;

  %PUT ***Syspbuff contains: &syspbuff***;
  %let num=1;
  %let respvar=%scan(&syspbuff,&num);
  %do %while(&respvar ne);

proc glimmix data=strat plots=studentpanel;
  class year block ccrop nitrogen; by depth;
  model &respvar = ccrop|nitrogen|year / ddfm=kr;
  random block block(ccrop);
  lsmeans ccrop|nitrogen|year / lines cl pdiff;

  %let num=%eval(&num+1);
  %let respvar=%scan(&syspbuff,&num);
  %end;
%mend mixanova;

%mixanova (pH P K NH4 NO3 TotalN_perc TotalC_perc OM_perc);

RUN; QUIT;
```